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Bactericidal properties of group IIa secreted phospholipase A_2 against *Pseudomonas aeruginosa* clinical isolates

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It has been shown that human group IIa secreted phospholipase A_2 ($sPLA_2$), found at high levels in inflammatory fluids, displays direct bactericidal properties against Gram-positive bacteria, while activity against Gram-negative bacteria requires the complement system or additional co-factors produced by neutrophils. *Pseudomonas aeruginosa*, an increasingly prevalent opportunistic human pathogen, is the most common Gram-negative rod found in cystic fibrosis lung infections, where it is associated with an inflammatory environment. Because murine intestinal group II $sPLA_2$ produced by Paneth cells has been shown to be directly bactericidal against Gram-negative bacteria, IIa $sPLA_2$ activity against *P. aeruginosa* clinical isolates was evaluated and provides the first evidence that the enzyme can be fully bactericidal in a concentration- and time-dependent manner against Gram-negative rods. Furthermore, it was demonstrated that these bactericidal properties were unaffected by high protein and salt concentrations, as observed in cystic fibrosis secretions, and that bacterial killing paralleled phospholipid hydrolysis. Finally, no cytotoxicity was observed when IIa $sPLA_2$ was incubated with human pulmonary cells, highlighting its potential use to synergize bactericidal antibiotics by promoting sublethal alterations of the bacterial cell wall.

INTRODUCTION

Phospholipases A_2 (PLA_2) cleave fatty acids from the *sn*-2 position of phospholipids from bilayers or micelles, yielding free fatty acids and lysophospholipids. They have been classified into several groups based on their secretory or cytosolic nature and their molecular structure. Group IIa secreted PLA_2 (IIa $sPLA_2$) has been attracting interest for almost two decades, since its discovery in platelets (Murakami *et al*., 1997) and synovial fluids and its association with inflammatory disorders (Valdas *et al*., 1993). Much effort has been expended to determine the precise role of $sPLA_2$ in physiological mechanisms. Interestingly, Weinrauch *et al*. (1996) demonstrated that, in infectious contexts, inflammatory exudates could exhibit potent antibacterial properties against both Gram-positive and Gram-negative bacteria. Furthermore, it has been demonstrated that, during systemic bacterial challenge, $sPLA_2$ is fully mobilized, conferring on plasma a potent bactericidal activity against *Escherichia coli* and *Staphylococcus aureus* (Weinrauch *et al*., 1998). In non-pathological circumstances, physiological fluids such as tears are naturally enriched in $sPLA_2$: concentrations as high as 60 μg ml^{-1} are frequently observed and are able to kill bacteria of the local flora such as *Micrococcus* species *in vitro* (Qu & Lehrer, 1998). More recently, studies involving transgenic mice expressing human group II $sPLA_2$ gene (PLA_2^+ mice) and group II PLA_2-deficient mice (PLA_2^- mice) allowed an accurate assessment of the antimicrobial role of group II $sPLA_2$ *in vivo*. For this purpose, Laine *et al*. (1999) evaluated the response of PLA_2^- mice versus PLA_2^+ mice when challenged by *Staphylococcus aureus* and noted an increased morbidity and mortality in the first group. In contrast, expression of $sPLA_2$ resulted in improved resistance of the animals by increased killing of bacteria, as indicated by the small number present in the tissues (Laine *et al*., 1999). Similar results were obtained

Abbreviations: BPI, bactericidal/permeability-increasing protein; CF, cystic fibrosis; $sPLA_2$, secreted phospholipase A_2.

when transgenic mice were challenged by *E. coli* (Laine *et al.*, 2000). These results emphasize the importance of $sPLA_2$ in the inflammatory response related to bacterial invasion and its major role in host defence.

Biochemical studies have shown that the bactericidal properties of $sPLA_2$ rely on cell-wall phospholipid hydrolysis. While destruction of Gram-positive rods has been mostly attributed to direct group IIa $sPLA_2$ activity, Gram-negative bacteria are considered to require both $sPLA_2$ and co-factors. These co-factors [e.g. complement system or antimicrobial peptides secreted by polymorphonuclear neutrophils such as bactericidal/permeability-increasing protein (BPI)] are thought to be responsible for an acute disruption of the bacterial envelope (Elsbach *et al.*, 1994). Nevertheless, the structurally related murine group II $sPLA_2$ secreted by Paneth cells of the intestine is able to display, on its own, effective bactericidal properties against Gram-negative pathogens such as *Salmonella typhimurium* and *E. coli* (Harwig *et al.*, 1995). Therefore, group II $sPLA_2$ is one of the first intestinal host-defence mechanisms.

Among Gram-negative rods, *Pseudomonas aeruginosa* is an increasingly prevalent opportunistic pathogen and is the most prominent rod isolated from cystic fibrosis (CF) lungs. In airway secretions, it is associated with increased inflammation and a highly salt-enriched microenvironment known to impair innate immunity components such as defensins (Smith *et al.*, 1996). Almost 80 % of CF patients beyond their teens are infected by *P. aeruginosa* and are more likely to die than patients with other kinds of pneumonia. Furthermore, *P. aeruginosa* is one of the few bacterial species that is naturally resistant to several β-lactam antibiotics and frequently evolves to multiresistant antibiotypes. Therefore, in the past decade, a great deal of effort has been put into attempts to make the *P. aeruginosa* outer membrane permeable to antibiotics in order to promote bactericidal activity.

The aim of the present work was to assess whether IIa $sPLA_2$ could display direct bactericidal properties against *P. aeruginosa* clinical isolates and to study the impact of the enzyme on the *P. aeruginosa* cell wall.

METHODS

Bacterial strains. *Staphylococcus aureus* ATCC 25923, *P. aeruginosa* ATCC 27583 and PAO1 and *Burkholderia cepacia* ATCC 25416^T were used as reference strains. Clinical isolates of the three species were obtained from the collection of the Bacteriology department of Rangueil Hospital (Toulouse, France) and their origins are listed in Table 1. All strains were stored in glycerol at −80 °C and were plated on blood agar before use.

Group IIa $sPLA_2$. Recombinant human group IIa $sPLA_2$ was produced in *E. coli* as described by Fourcade *et al.* (1995).

Assay for bactericidal activity. Bacteria were grown overnight at 37 °C in trypticase soy broth (TSB) and then diluted 1 : 10 in fresh medium and subcultured to mid-exponential phase. After harvesting, the bacteria were sedimented by centrifugation at 14 000 ***g*** for 5 min, washed twice with 10 mM HEPES/NaOH (pH 7·6), 0·15 M NaCl, 10 mg BSA ml^{-1} and 2·5 mM $CaCl_2$ and resuspended in the same solution at a concentration of 10^8 c.f.u. ml^{-1}.

Bactericidal activity was assayed as follows. Bacterial suspensions (10^8 c.f.u.) were incubated with appropriate dilutions (8, 16 and 32 μg ml^{-1}) of group IIa $sPLA_2$ in the saline buffer described previously containing either BSA or serum. Mixtures were incubated at 37 °C with shaking for

Table 1. Characteristics of the strains tested

Abbreviations: CF, cystic fibrosis; MR, multiresistant strain.

Strain	Origin	Characteristics
Staphylococcus aureus		
ATCC 25923	Clinical isolate	Reference strain
0412	CF sputum	Chronic colonization; MR
Pseudomonas aeruginosa		
ATCC 27583	Blood culture	Reference strain
PAO1	Infected wound	Reference strain
7844S	CF sputum	Child; chronic colonization; MR (smooth)
7844M	CF sputum	Child; chronic colonization; MR (mucoid revertant of 7844S)
0310S	CF sputum	Child; chronic colonization; MR (smooth)
0310M	CF sputum	Child; chronic colonization; MR (mucoid revertant of 0310S)
94	CF sputum	Primocolonization (smooth)
96	CF sputum	Two-year colonization (smooth)
98	CF sputum	Adult; chronic colonization; MR (mucoid)
99	CF sputum	Adult; chronic colonization; MR (mucoid)
Burkholderia cepacia		
ATCC 25416^T	Environment	Reference strain
235	CF sputum	Primocolonization

1 or 3 h and then filtered on 0·20 μm Millipore filters. Membranes were then plated onto trypticase soy agar (TSA) and grown at 37 °C for 18 h in an aerobic atmosphere.

The bactericidal effect of group IIa $sPLA_2$ was expressed as the residual number of c.f.u. with respect to the initial inoculum and the EC_{50} (concentration eliciting 50 % effect) corresponding to the enzyme concentration able to kill 50 % of the initial inoculum. The results presented are means of at least three independent experiments.

Effects of salt concentration and proteins on bactericidal effects of $sPLA_2$. The bactericidal properties of $sPLA_2$ were tested in the presence of various incubation media. These media were BSA in the range 0 to 10 mg ml^{-1} and NaCl from 0 to 0·25 M. Results were expressed as means ± SD of triplicate determinations. Statistical analysis was then performed using a paired *t*-test.

Radiolabelling of bacterial lipids. Bacteria were subcultured to mid-exponential phase in TSB supplemented with 2 μCi [1-^{32}P]oleic acid ml^{-1} (Amersham Lifescience France) (1 Ci = $3{\cdot}7 \times 10^7$ Bq) and 0·1 % BSA (w/v). Bacteria were then harvested and cultured in the same medium without [1-^{32}P]oleic acid at 37 °C for 30 min in order to remove unesterified fatty acid precursor incorporated into ester positions. Finally, the bacteria were washed with saline buffer.

Lipid analysis. Bacteria (10^8 c.f.u.) were exposed to 5 or 10 μg $sPLA_2$ ml^{-1} for up to 3 h. The composition of the lipids was then determined by extraction in $CHCl_3/CH_3OH$ as described by Bligh & Dyer (1959) and analysis of the radiolabelled material recovered in the $CHCl_3$ phase (> 95 % of total) by TLC in the presence of standards. Results were expressed as the percentage hydrolysis of phospholipids compared with a control performed in the absence of the enzyme. Data are means ± SD of three independent experiments.

Assay for cytotoxicity to human pulmonary cells. In order to test the possible cytotoxic effect of group IIa $sPLA_2$ on human cells, the viability of tracheal epithelial cells incubated with an appropriate dilution of the enzyme was evaluated for 24 h.

Immortalized and characterized tracheal epithelial cells from fetuses were a gift from URA CNRS 1283 (Paris, France) (Lemnaouar *et al.*, 1993). Cells were grown to confluence at 37 °C under 5 % CO_2 in 1 : 1 DMEM/Ham's F12 medium supplemented with 2 % (w/v) Ultroser G, 100 IU penicillin ml^{-1} and 100 μg streptomycin ml^{-1}.

Solutions containing 8, 16 or 32 μg $sPLA_2$ ml^{-1} were mixed with DMEM while the same volume of saline buffer was used as a negative control. A 10 % (v/v) solution of Triton X-100 in DMEM was used as a positive control. At 0, 6, 12 and 24 h, the supernatant was carefully removed and centrifuged at 14 000 ***g*** for 5 min in order to remove cells. Lactate dehydrogenase (LDH) in the supernatant was evaluated and a ratio $LDH_{assay}/LDH_{positive\ control}$ > 5 % was considered to indicate cytotoxicity. The cytotoxicity test was performed three times for each treatment.

RESULTS

Group IIa $sPLA_2$ is able to kill *P. aeruginosa* clinical isolates in a concentration-dependent manner

In order to determine whether group IIa $sPLA_2$ could display antibacterial properties against *P. aeruginosa* clinical isolates, viable c.f.u. were evaluated after incubation of 10^8 c.f.u. ml^{-1} with various concentrations of $sPLA_2$ for 1 h. Since the enzyme was recombinant, we decided to control its activity against *Staphylococcus aureus* strains, which are known to be susceptible, in parallel. Under these experimental conditions, a marked reduction of the inoculum was observed for both species within 1 h for concentrations as low as 8 μg ml^{-1} (Fig. 1). A progressive reduction of approximately 90 % of the initial inoculum was observed for all *P. aeruginosa* strains when concentrations were increased gradually up to 32 μg ml^{-1} (data not shown). Interestingly, as listed in Table 2, comparable bactericidal properties were observed for both smooth and mucoid strains, which displayed similar EC_{50} after 1 h of incubation. However, two CF strains isolated from adult patients required larger amounts of $sPLA_2$. In contrast, little or no $sPLA_2$ activity could be observed against the two *B. cepacia* strains tested. It is interesting to note that the range of concentrations necessary to observe a bactericidal effect was almost identical for the *Staphylococcus aureus* and *P. aeruginosa* strains tested. Furthermore, these results are the first evidence of direct activity of group IIa $sPLA_2$ against a Gram-negative species without the addition of any co-factor.

Group IIa $sPLA_2$ bactericidal activity is time-dependent

In order to investigate whether bactericidal activity could be enhanced by longer incubation, all strains were subjected to $sPLA_2$ for up to 3 h. Table 2 lists the relative EC_{50} values for bactericidal activity against all the strains tested. Recombinant $sPLA_2$ was thus more effective against both *Staphylococcus aureus* and *P. aeruginosa* strains when the incubation time was increased. The minimal concentrations required for EC_{50} were mostly close to 2–8 μg ml^{-1} when incubated for 3 h, compared with 5–12 μg ml^{-1} after only 1 h. These results suggest that bactericidal activity is time-dependent and that increased contact between enzyme and bacterial strains, as would occur under physiological conditions, dramatically enhances the antibacterial properties of $sPLA_2$.

Group IIa $sPLA_2$ bactericidal properties are not affected by high concentrations of protein or NaCl

In order to test whether the abnormal composition of CF airway secretions could affect $sPLA_2$, we tested whether the enzyme bactericidal properties could be modified by protein and salt variations. As shown in Table 3, similar bactericidal effects were observed for concentrations as high as 32 μg $sPLA_2$ ml^{-1}, whatever the test conditions; no significant difference was observed either when the protein content was increased or when high salt concentrations were added to the incubation medium ($P > 0{\cdot}05$). When human serum was used instead of BSA, no inhibitory effect could be observed (data not shown). These results demonstrate that, even if an unfavourable microenvironment is present, as observed in CF lungs, $sPLA_2$ is able to display potent bactericidal activity against both *Staphylococcus aureus* and *P. aeruginosa* strains.

Group IIa $sPLA_2$ bactericidal activity parallels phospholipid hydrolysis

In order to evaluate whether the antimicrobial properties of group IIa $sPLA_2$ against *P. aeruginosa* paralleled significant

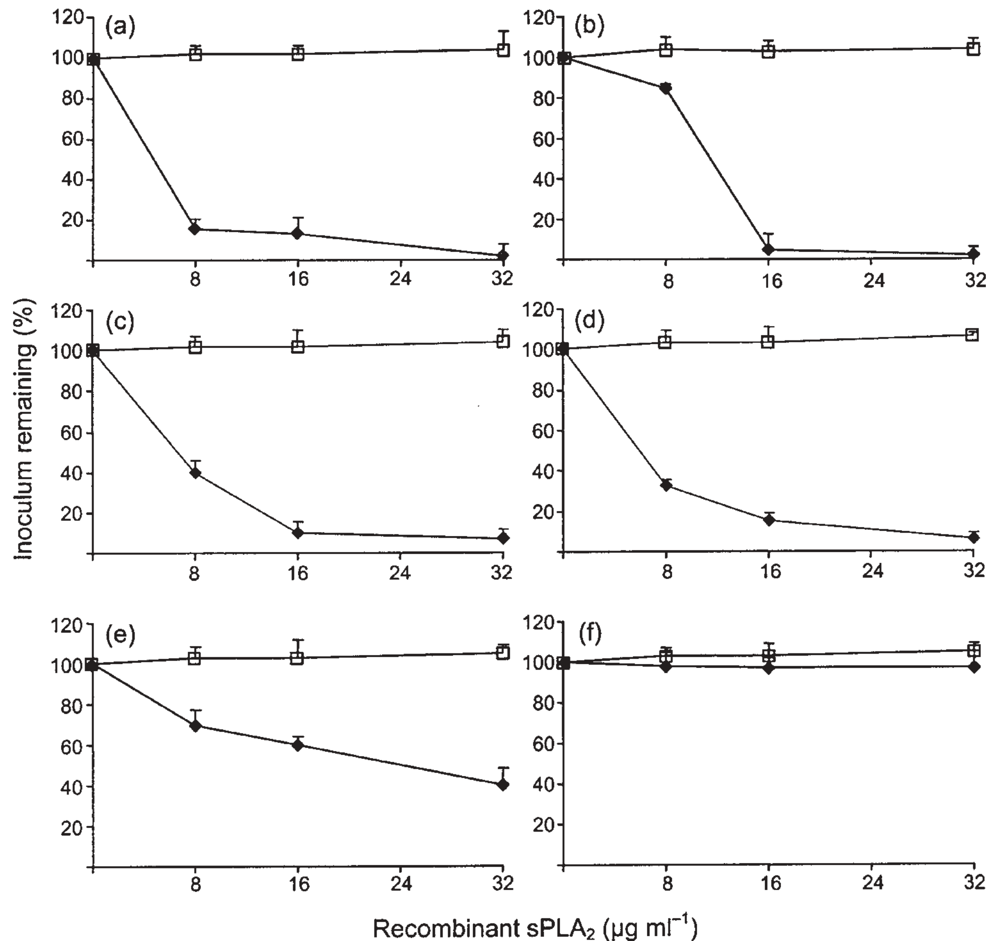

Fig. 1. Recombinant human $sPLA_2$ is able to kill *Staphylococcus aureus* and *P. aeruginosa* strains *in vitro*. Samples were incubated for 60 min with 8, 16 and 32 µg $sPLA_2$ (◆) ml^{-1} or saline buffer (□) and then grown on TSA. Bactericidal effect was evaluated by counting c.f.u. after 24 h and is expressed as the percentage of inoculum remaining (c.f.u.%). Strong activity was observed for both *Staphylococcus aureus* strains ATCC 25923 (a) and 0412 (b) and *P. aeruginosa* strains ATCC 27583 (c) and 7844 (d) over the same range of $sPLA_2$ concentrations, while *B. cepacia* strains ATCC 25416^T (e) and 235 (f) were more resistant. Data shown are means ± SD ($n = 3$).

hydrolysis of the bacterial phospholipids, as demonstrated for *Staphylococcus aureus* strains, the ability of the enzyme to liberate oleic acid from the bacterial membrane was also studied.

As shown in Fig. 2, incubation of radiolabelled bacteria with 5 µg $sPLA_2$ ml^{-1} led to the degradation of 60 % of the major phospholipids within 3 h. Furthermore, increased degradation of phospholipids was observed for higher concentrations (10 µg ml^{-1}). These results indicate that the ability of IIa $sPLA_2$ to hydrolyse phospholipids of the bacterial envelope correlates with the concentration- and time-dependent bactericidal activity.

Group IIa $sPLA_2$ is not cytotoxic to human cells

Human tracheal epithelial cells were incubated with saline buffer or $sPLA_2$ concentrations identical to those used to display bactericidal activity in order to investigate possible cytotoxic effects. As shown in Table 4, for all samples, the ratio $LDH_{assay}/LDH_{positive\ control}$ was <5 %, even after 24 h incubation and was considered not to indicate cytotoxicity. These results show that $sPLA_2$ concentrations able to display bactericidal activity are totally innocuous to human pulmonary cells.

DISCUSSION

P. aeruginosa is an increasingly prevalent opportunistic pathogen and is frequently isolated from CF chronic lung infections, which are responsible for considerable morbidity in over 80 % of patients (Cystic Fibrosis Foundation, 2001). *P. aeruginosa* chronic infection is essentially characterized by the emergence of mucoid strains that produce an exopolysaccharide coat, which confers resistance to phagocytosis and antibiotics and is responsible for major obstruction of the airways (Doggett *et al.*, 1966). Furthermore, *P. aeruginosa*

Table 2. sPLA$_2$ displays time-dependent antimicrobial activity against *Staphylococcus aureus* and *P. aeruginosa* strains

Bacterial strains were incubated with various concentrations (8, 16 or 32 μg ml^{-1}) of sPLA$_2$ for 1 or 3 h. The bactericidal effect of the enzyme was assessed by the enzyme concentration necessary to kill 50 % of the initial inoculum (EC$_{50}$), which was deduced from curves obtained from three independent experiments.

Strain	EC$_{50}$ (μg ml^{-1})	
	1 h	3 h
Staphylococcus aureus		
ATCC 25923	5	2
0412	12	8
P. aeruginosa		
ATCC 27853	6	2
PAO1	7	4
7844S	6	3
7844M	6	3
0310S	6	3
0310M	6	3
94	7	4
96	8	4
98	12	8
99	16	9
B. cepacia		
ATCC 25416^T	18	16
235	> 32	> 32

Table 3. Effects of protein (BSA) and NaCl on bactericidal properties of sPLA2 against *P. aeruginosa*

P. aeruginosa ATCC 29583 (10^8 c.f.u. ml^{-1}) was incubated at 37 °C with 32 μg recombinant human sPLA$_2$ ml^{-1} supplemented with various concentrations of BSA and NaCl (M) as described in Methods. After 60 min, bacterial viability was measured as described in Methods. Results are expressed as percentage viability with respect to bacteria incubated without sPLA$_2$ and are means of three independent experiments.

Supplementation		Viability (%)
BSA (mg ml^{-1})	NaCl (M)	
0	0·15	4 ± 0·2
5	0·15	3 ± 0·4
10	0·15	3 ± 0·1
10	0	4 ± 0·3
10	0·15	2 ± 0·5
10	0·25	3 ± 0·2

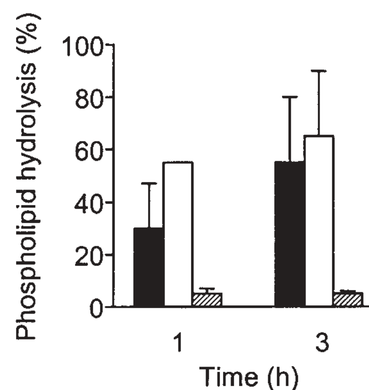

Fig. 2. Recombinant type IIa sPLA$_2$ bactericidal activity against *P. aeruginosa* strains parallels bacterial phospholipid envelope hydrolysis. *P. aeruginosa* ATCC 27583 was metabolically labelled in TSB supplemented with [1-^{32}P]oleic acid and 0·1 % (w/v) BSA, chased with non-radioactive TSB and then incubated with 5 (filled bars) or 10 (open bars) μg recombinant sPLA$_2$ ml^{-1} or without any enzyme (hatched bars). Bacterial phospholipid hydrolysis was determined by TLC and liquid scintillation counting as described in Methods. Results correspond to the percentage of phospholipid hydrolysis with respect to an untreated control. Data are means ± SD of three experiments.

Table 4. Viability of human tracheal epithelial cells incubated with sPLA$_2$

Cells were grown in DMEM supplemented with saline buffer (negative control), IIa sPLA$_2$ or Triton X-100 (positive control). Supernatants were removed carefully and lysis was assessed by the ratio LDH$_{assay}$/LDH$_{positive\ control}$, expressed below as a percentage. A ratio >5 % was considered to indicate cytotoxicity.

Incubation (h)	Saline buffer	IIa sPLA$_2$ (μg ml^{-1})			Triton X-100
		8	16	32	
0	< 1	< 1	< 1	< 1	< 1
6	< 1	1	1	1	100
12	< 1	1	2	2	100
24	1	3	2	3	100

infection is accompanied by an acute inflammatory response and altered antimicrobial peptide activity due to the highly salt-enriched lung secretions (Smith *et al.*, 1996). The ineffectiveness of innate host-defence mechanisms is clearly a major clinical problem for CF patients. Furthermore, this bacterium is intrinsically resistant to many antibiotics. Part of this resistance can be attributed to the relatively low permeability of the *P. aeruginosa* outer membrane to a variety of antibiotics (Hancock & Wong, 1984; Yoshimura & Nikaido, 1982). Another part of the resistance appears to be caused by multidrug efflux systems (Poole *et al.*, 1996a, b). Finally, in some cases, enzymes that specifically inactivate β-lactam antibiotics, e.g. inducible cephalosporinase or imipenemase of *P. aeruginosa* (Giwercman *et al.*, 1990; Livermore, 1987; Richmond & Sykes, 1973), can be secreted. Hence, over the past decade, a great deal of effort has gone

into attempts to make the Gram-negative outer membrane permeable to antibiotics and to deliver drugs as close as possible to the target, e.g. by using tobramycin nebulization.

In this report, we have examined the effects of recombinant IIa $sPLA_2$ on the viability of *P. aeruginosa* clinical isolates. Several investigators had shown previously that IIa $sPLA_2$, initially described as part of the inflammatory response, also displays bactericidal activity against Gram-positive bacteria such as *Staphylococcus aureus*, while activity against *E. coli* strains requires co-factors. The latter include the complement system or BPI and p15s, produced by neutrophils (Weinrauch *et al*., 1995), which lead to synergistic disruption of the bacterial envelope. Nevertheless, the closely related murine type II $sPLA_2$ produced by Paneth cells is able to exert a direct antimicrobial effect against Gram-negative rods (Harwig *et al*., 1995).

The first step in our study was to determine recombinant IIa $sPLA_2$ activity against *P. aeruginosa* in parallel with *Staphylococcus aureus* strains in order to validate the efficacy of the recombinant enzyme. We found that IIa $sPLA_2$ could be fully bactericidal against the *P. aeruginosa* and *Staphylococcus aureus* clinical isolates in a concentration-dependent fashion and at a similar range of concentrations for both species without the addition of any co-factors. To our knowledge, these results provide the first demonstration that IIa $sPLA_2$ can be intrinsically microbicidal to a Gram-negative species. It is interesting to note that $sPLA_2$ displayed comparable properties against both smooth and mucoid strains, indicating that bacterial alginate does not necessarily interfere with the bactericidal properties of the enzyme. Furthermore, most of the *P. aeruginosa* strains tested expressed antibiotic-multiresistant phenotypes, which did not interfere with their susceptibility to $sPLA_2$.

The abnormal composition of CF airway secretions is known to affect the action of antimicrobial peptides, since antimicrobial function is often dependent on ion strength and salt sensitivity is crucial for cationic peptides (Smith *et al*., 1996). We therefore tested the effects of protein and salt variations as well as acidic pH (data not shown) in order to mimic the CF lung microenvironment. No difference in activity was found among the various groups. This is of particular interest because it shows that, unlike many host-defence antimicrobial peptides. which are totally ineffective in CF because of airway secretion hyperosmolarity, $sPLA_2$ remains active.

We finally decided to evaluate the time-course of bactericidal properties and demonstrated that incubation for up to 3 h significantly enhanced the reduction of the initial inoculum. Taken together, these results show that IIa $sPLA_2$ can be an effective microbicide, the direct antibacterial potency of which against *P. aeruginosa* approximates to that against *Staphylococcus aureus* even under unfavourable conditions close to those observed in CF lungs.

We also observed that the enzyme was responsible for phospholipid hydrolysis and concomitant liberation of free fatty acids, as already demonstrated for Gram-positive bacteria. Indeed, incubation of radiolabelled bacteria with 5 μg $sPLA_2$ ml^{-1} led to degradation of 60 % of the major phospholipids within 3 h. This observation is consistent with the amount expected (e.g. 50 %) when only the phospholipids of the extracellular leaflet of the bilayered membrane (which is accessible to $sPLA_2$) are hydrolysed (Koduri *et al*., 2002), and is in agreement with observations on *Micrococcus luteus* strains (Buckland *et al*., 2000). Moreover, the degree and time-course of phospholipid hydrolysis correlated well with bacterial viability, even for mucoid and antibiotic-multiresistant strains.

These findings suggest that IIa $sPLA_2$ is intrinsically able to cause disruption of bacterial envelope integrity, thus leading to impairment of bacterial viability. One could then imagine that the enzyme could potentially synergize the effects of antibiotics such as β-lactam in combination with natural antibacterial peptides such as BPI. Because no cytotoxicity could be observed for concentrations comparable to those necessary to display bactericidal activity against *P. aeruginosa* strains, IIa $sPLA_2$ may be considered as a potential therapeutic agent synergizing antibiotics. However, one of the main problems when administering antibiotics to CF patients is how to achieve targeted drug delivery to the diseased tissue in order to obtain microbicidal concentrations as high as possible while limiting side effects. Therefore, new administration routes such as tobramycin nebulization have been proposed in recent years (Doring *et al*., 2000). In this context, the dual potency of IIa $sPLA_2$ to hydrolyse not only bacterial phospholipids but also micelles of phospholipid bilayers should be of particular interest. Hence, new targeting systems such as liposomal drug-carrier systems are being developed. The action of IIa $sPLA_2$ on liposomes can be used to release their molecular content (e.g. drugs) directly where needed. This method has in fact been demonstrated to be fully efficient for anti-cancer drug delivery, while tumorous tissues are known to hyperexpress IIa $sPLA_2$ (Davidsen *et al*., 2003). One could easily imagine that this method could be used in CF therapy.

Additional work is obviously needed to clarify the various possible strategies. Nevertheless, these findings may prove interesting with regards to new therapeutic strategies, particularly since more drug-resistant bacterial strains are emerging.

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