

Antibacterial effects of human group IIA and group XIIA phospholipase A2 against *Helicobacter pylori* in vitro

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Group IIA phospholipase A2 (PLA2-IIA) is an enzyme which has important roles in inflammation and infection. Recently, a novel human secretory PLA2 called group XIIA PLA2 (PLA2-XIIA) has been identified. Both PLA2-IIA and PLA2-XIIA are bactericidal against Gram-positive bacteria like many other secretory PLA2s. However, PLA2-XIIA is the only known PLA2 displaying significant bactericidal activity against the Gram-negative bacterium *Escherichia coli*. We examined the antibacterial properties of recombinant human PLA2-IIA and PLA2-XIIA against *Helicobacter pylori*, a Gram-negative bacterium, *in vitro*. PLA2-IIA was not bactericidal against *H. pylori*, whereas PLA2-XIIA effectively killed *H. pylori* at a concentration of 50 µg/ml but was not bactericidal at concentrations of 0.5 µg/ml and 5 µg/ml.

Key words: Group IIA phospholipase A2; group XIIA phospholipase A2; *Helicobacter pylori*; infection; inflammation; *in vitro*.

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Helicobacter pylori is a Gram-negative bacterium measuring 2.5–4 µm and having a flat spiral shape. It is a significant human gastroduodenal pathogen and has been associated with peptic ulcer disease, gastric mucosa-associated lymphoid tissue lymphoma, and gastric cancer (1).

Phospholipase A2 (PLA2) is a lipolytic enzyme that hydrolyzes phospholipids into free fatty acids and corresponding lysocompounds. The superfamily of PLA2 enzymes consists of several groups, of which group IIA PLA2 (PLA2-IIA) is the best known (2). The concentration of PLA2-IIA increases in sera of patients suffering from severe acute inflammatory

diseases, such as sepsis and bacterial infections (3) and acute pancreatitis (4). PLA2-IIA is an acute-phase protein and an important factor in the host defence against invading bacteria (5–8). PLA2-IIA is highly bactericidal against Gram-positive bacteria (6, 9). This antibacterial potential is due to its capacity to degrade bacterial phospholipids by its catalytic activity and by perturbing the bacterial cell wall with its high cationic properties (10). In comparison, the bactericidal potency of PLA2-IIA against *Escherichia coli* and other Gram-negative microbes is minimal (6) and requires the action of other proteins, e.g. bactericidal/permeability-increasing protein (BPI) (5) and complement (11).

Recently, a novel human secretory PLA2 called group XIIA PLA2 (PLA2-XIIA) was identified and strong expression of this enzyme

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was found in the heart, skeletal muscle, kidney, and pancreas (12). PLA2-XIIIA is not cationic and also has a low catalytic activity compared to other mammalian secreted PLA2s (12–14). Group XIIIA PLA2 is structurally distinct from other secretory PLA2s and may therefore have other functions besides its lipolytic activity (13). PLA2-XIIIA, like many other secretory PLA2s, has been shown to be bactericidal against Gram-positive bacteria *in vitro*. In addition, PLA2-XIIIA is the only known PLA2 displaying detectable bactericidal activity against the Gram-negative bacterium *E. coli in vitro* (13).

The purpose of the present study was to examine the antibacterial properties of recombinant human PLA2-IIA and PLA2-XIIIA against the Gram-negative bacterium *H. pylori in vitro*.

MATERIALS AND METHODS

Recombinant human group IIA and XIIIA phospholipase A2

Human PLA2-IIA used in this study was obtained by bacterial expression and *in vitro* refolding as described earlier (14, 15). Human PLA2-XIIIA was produced by recombinant expression in *E. coli* as described earlier (13). For the antibacterial studies, PLA2-IIA was used at a concentration of 50 µg/ml and PLA2-XIIIA at concentrations of 0.5 µg/ml, 5 µg/ml and 50 µg/ml (13).

H. pylori cultures

The *H. pylori* strain ATCC 43503 was used in this study. The organisms were grown for 48 h at 37°C under microaerobic conditions on 5% lysed horse blood agar supplemented with antibiotics. Thereafter, the bacteria were scraped from the agar and suspended in 2 ml of brain heart infusion broth (BHIB), and the OD at 650 nm (OD₆₅₀) of the suspension was adjusted to ~1.300 by diluting with BHIB. This value is equivalent to ~8×10⁸ bacteria/ml. OD₆₅₀ was measured with an Ultrospec III spectrophotometer (Pharmacia, Piscataway, NJ, USA). A

total of 20 µl of the suspension was added to 990 µl of HEPES buffer (20 mmol/l HEPES, 2.0 mmol/l Ca²⁺, 10 mg/ml BSA, pH 7.4) and 990 µl BHIB, and shaken. The 0 min sample was taken from this dilution. Fifteen microliters of this suspension was added to tubes with 15 µl of PLA2-suspension (20 mmol HEPES+PLA2) or 15 µl of BHIB as control. After centrifugation (500×g, 5 s) the tubes were incubated at 37°C, and 5 µl samples were taken at 20, 60, and 120 min. The number of live bacteria in the growth medium at each time point was determined by diluting the samples in sterile saline at logarithmic scale and placing them on 5% lysed horse blood agar at 37°C under microaerobic conditions. The bacterial colonies were counted at 7 days. The bactericidity tests were performed twice.

RESULTS

The results are shown in Table 1, which presents the survival of *H. pylori* as percentage viable *H. pylori* bacteria at each time point compared to the original number of bacteria. The value 100% represents the number of bacteria at the beginning of the incubation (0 min). The most conspicuous finding is that PLA2-XIIIA, at a concentration of 50 µg/ml, killed all *H. pylori* bacteria. Neither PLA-XIIIA at concentrations 0.5 µg/ml and 5 µg/ml nor PLA2-IIA at a concentration of 50 µg/ml were bactericidal against *H. pylori*.

DISCUSSION

PLA2-IIA has been shown to be effectively bactericidal against Gram-positive bacteria *in vitro* (6, 9). However, the bactericidal action of PLA2-IIA against Gram-negative bacteria is more complex. To kill *E. coli* bacteria, PLA2-IIA requires the synergistic action of BPI, an antimicrobial protein produced by polymorphonuclear leukocytes (5), and PLA2-IIA is ineffec-

TABLE 1. Survival of *Helicobacter pylori* in the presence of PLA2-IIA and PLA2-XIIIA. The data presented here represent percentage of viable bacteria colony-forming units compared to the number of bacteria at the beginning of the incubation (0 min). The numeric data represent mean values of two independent tests

Time (min)	PLA2-IIA		PLA2-XIIIA		Control
	50 µg/ml	0.5 µg/ml	5 µg/ml	50 µg/ml	
20	54	60	80	0	55
60	40	48	52	0	40
120	27	32	48	0	24

tive against *E. coli* without BPI (11). The antibacterial properties of secretory PLA2s against *H. pylori* have not been studied previously. Our current results show that PLA2-IIA has no bactericidal effect on *H. pylori in vitro*.

Human PLA2-XIIA is the only secretory PLA2 with bactericidal activity against the Gram-negative bacterium *E. coli in vitro* (13). The potency of PLA2-XIIA against *E. coli* was relatively weak at concentrations of 0.5 µg/ml, 5 µg/ml and 50 µg/ml (13). In the present study, PLA-XIIA killed all *H. pylori* bacteria at a concentration of 50 µg/ml but had no detectable bactericidal effects at concentrations 5 µg/ml and 0.5 µg/ml. Therefore, it seems that only high concentrations of PLA2-XIIA are bactericidal against *H. pylori* and it is possible that the bactericidal activity of PLA2-XIIA is not due to its lipolytic activity. Some human cell lines exhibit abnormal morphology when transfected with PLA2-XIIA, suggesting a role of this enzyme in cell division (16). The mechanism, lipolytic or other, by which PLA2-XIIA kills *H. pylori* remains to be established.

The viability of *H. pylori* seemed to be reduced with time in all surviving groups in the present study. The incubation of bacteria in PLA2-containing solutions was performed in room air containing 21% oxygen, which is not optimal for *H. pylori*, since the bacteria prefer low oxygen levels. After incubation, the growth medium was placed in microaerophilic conditions. Therefore, the lower percentage of living bacteria at later time points in the current study may be due to the unfavourable living conditions during incubation.

In conclusion, the current results show that PLA2-XIIA is bactericidal against *H. pylori in vitro*, whereas PLA2-IIA is ineffective.

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