Biophysical Studies Reveal Key Interactions between Papiliocin-Derived PapN and Lipopolysaccharide in Gram-Negative Bacteria

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Papiliocin, isolated from the swallowtail butterfly (*Papilio xuthus*), is an antimicrobial peptide with high selectivity against gram-negative bacteria. We previously showed that the N-terminal helix of papiliocin (PapN) plays a key role in the antibacterial and anti-inflammatory activity of papiliocin. In this study, we measured the selectivity of PapN against multidrug-resistant gram-negative bacteria, as well as its anti-inflammatory activity. Interactions between Trp2 of PapN and lipopolysaccharide (LPS), which is a major component of the outer membrane of gram-negative bacteria, were studied using the Trp fluorescence blue shift and quenching in LPS micelles. Furthermore, using circular dichroism, we investigated the interactions between PapN and LPS, showing that LPS plays critical roles in peptide folding. Our results demonstrated that Trp2 in PapN was buried deep in the negatively charged LPS, and Trp2 induced the α -helical structure of PapN. Importantly, docking studies determined that predominant electrostatic interactions of positively charged arginine residues in PapN with phosphate head groups of LPS were key factors for binding. Similarly, hydrophobic interactions by aromatic residues of PapN with fatty acid chains in LPS were also significant for binding. These results may facilitate the development of peptide antibiotics with anti-inflammatory activity.

Keywords: Antimicrobial peptide, PapN, inflammation, LPS, antibiotics

Introduction

Antimicrobial peptides (AMPs) play important roles in the innate immunity of various organisms [1, 2]. Cecropin was the first α -helical AMP to be isolated from the hemolymph of bacteria-challenged, diapausing pupae of the giant silk moth *Hyalophora cecropia*. Subsequently, several cecropins with a length of 31 to 42 amino acids were identified from other insects [3, 4]. These compounds are highly cationic and have conserved aromatic residues located at the N-terminus. Papiliocin is an insect cecropin with 37 residues (RWKIFKKIEKVGRNVRDGIKAGPAVAVVGQAATVVK-NH₂) found in the larvae of the swallowtail butterfly *Papilio xuthus* and exhibits potent antibacterial activity against gram-negative bacteria as well as antifungal activity [5–7]. The three-dimensional structure of papiliocin was determined using nuclear magnetic resonance (NMR) spectroscopy, which revealed an

amphipathic N-terminal α -helix, a C-terminal hydrophobic α -helix, and a hinge in between [6]. Our previous studies showed that substitution of Trp2 with Ala in papiliocin proved that Trp2 plays a critical role in the antibacterial activity of papiliocin. The N-terminal helical domain of papiliocin (PapN; RWKIFKKIEKVGRNVRDGIKA) possessed substantial antibacterial and anti-inflammatory activities, whereas the C-terminal region did not show antibacterial activity [8, 9].

Several studies have focused on peptides with anti-inflammatory activity that can act as potential candidates against sepsis, infection, and other inflammatory stimuli [10]. Importantly, many AMPs are known to interact with lipopolysaccharide (LPS), which stimulates inflammation during infection by gram-negative bacteria [11]. For example, the cathelicidin peptide LL-37 is highly effective at inhibiting LPS-induced cellular cytokine and nitric oxide (NO) released by binding directly to LPS or blocking the

binding of LPS to LPS-binding protein [12, 13]. It has been reported that patients lacking LL-37 are more susceptible to bacterial infections [14]. Sepsis, which originates from a pathogenic infection, causes a serious LPS-mediated inflammatory response [15]. A dysfunction of the host immune defense system against pathogenic infection might be the major reason for sepsis. This has sparked a growing search for antibiotics to treat sepsis [16].

In this study, we explored the interactions between LPS and PapN, a possible source of short antibiotics. The anti-inflammatory activity of PapN was investigated by examining its inhibition of NO and cytokine production in LPS-stimulated RAW264.7 cells. To understand the origin of the antibacterial and anti-inflammatory activities of PapN, we investigated the direct interaction between PapN and LPS using circular dichroism (CD) and tryptophan fluorescence studies. We also built a binding model of PapN and LPS using molecular docking study, further proving that PapN could be a potent anti-inflammatory agent.

Materials and Methods

Peptide Synthesis

PapN and other peptides were synthesized by solid-phase synthesis as described previously [17]. Peptides were purified with a purity higher than 95% by reversed-phase preparative high-performance liquid chromatography. We determined the molecular masses of the peptides using a matrix-assisted laser-desorption ionization-time-of-flight mass spectrometer at the Korea Basic Science Institute (Ochang, Korea).

Antibacterial Activity

The minimal inhibitory concentration (MIC) for each of the peptides against six gram-negative drug-resistant bacteria (*Escherichia coli* CCARM 1229, *E. coli* CCARM1238, *Salmonella* serovar Typhimurium CCARM 8003, *S. serovar* Typhimurium CCARM 8007, *Acinetobacter baumannii* CCARM12035, and *A. baumannii* CCARM12010) and two gram-positive drug-resistant bacteria (*Staphylococcus aureus* CCARM 3089 and *S. aureus* CCARM 3090) was determined using the broth microdilution method as described previously [17]. The lowest concentration of peptides that completely inhibited the growth of bacteria was considered as the MIC.

Cytotoxicity

Mouse fibroblast NIH3T3 cells were cultured in a 5% CO₂ atmosphere using Dulbecco's modified Eagle's medium supplemented with 10% heat-inactivated fetal bovine serum and antibiotics (100 U/ml penicillin, 100 µg/ml streptomycin). The percentage of growth inhibition was evaluated using a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay

for the measurement of viable cells as described previously [18].

Tryptophan Fluorescence Blue Shift

We prepared small unilamellar vesicles (SUVs) using egg yolk PC/PG (EYPC/EYPG) (7:3 (w/w)) as described previously [6]. SUVs were added to a peptide solution (5 µM final concentration) in 10 mM Tris buffer (pH 7.4), 0.1 mM ethylenediamine tetraacetic acid, and 150 mM NaCl. Trp2 from the peptide was excited at 280 nm, and emission spectra were recorded at 300–400 nm using a 5-nm band pass filter. Blue shifts were measured also in 10 mM sodium phosphate buffer.

Quenching of Tryptophan Fluorescence by Acrylamide

A peptide solution was added to LPS to maintain the LPS:peptide molar ratio. Excitation of Trp2 was performed at 295 nm instead of 280 nm to decrease the absorbance contributed by acrylamide [6]. Tryptophan fluorescence was quenched by titration with acrylamide, from a 4 M stock solution, to the final concentration of 0.4 M in the presence of LPS at a peptide:lipid molar ratio of 1:60. The quenching data were analyzed by using the Stern-Volmer plot with the equation $F_0/F = 1 + K_{sv}[Q]$, where F_0 is the fluorescence of the peptide without acrylamide, F is the fluorescence of the peptide with acrylamide, K_{sv} is the Stern-Volmer quenching constant, and $[Q]$ is the concentration of acrylamide.

Nitric Oxide Production in LPS-Stimulated RAW264.7 Cells

Mouse RAW264.7 macrophages (1×10^5) were plated in each well of 96-well culture plates and stimulated with LPS (20 ng/ml) from *E. coli* O111:B4 (Sigma-Aldrich, USA) in the presence or absence of peptides. Nitrite accumulation in the culture medium was used as an indicator of NO production [19]. Nitrite concentrations were calculated with a standard curve generated using NaNO₂ as described previously [20].

Quantification of Cytokine TNF-α Production

Antibody for mouse tumor necrosis factor-α (mTNF-α) was immobilized on immunoplates by incubating with 0.2–0.8 µg/ml solutions of antibody overnight at room temperature. Peptide-induced inhibition of TNF-α in LPS-stimulated RAW264.7 cells was determined as described previously [17]. Quantification of TNF-α production was determined by enzyme-linked immunosorbent assay (R&D Systems, USA).

Circular Dichroism

CD experiments were performed using a J810 CD spectropolarimeter (Jasco, Japan) at 0.1-nm intervals from 190 to 250 nm. CD spectroscopy was used to investigate the secondary structural changes of 50 µM peptides following addition of 0–0.4 mg/ml LPS.

Prediction of Binding Model

For molecular docking of PapN/LPS, AutoDock Vina

Table 1. Antibacterial activity of papiliocin and PapN against drug-resistant bacteria.

Peptides	MIC (μM)							
	Gram-negative				Gram-positive			
	<i>E. coli</i> 1229	<i>E. coli</i> 1238	<i>S. Typhimurium</i> 8007(R)	<i>S. Typhimurium</i> 8003(R)	<i>A. baumannii</i> 112035(R)	<i>A. baumannii</i> 12010(R)	<i>S. aureus</i> 3089(R)	<i>S. aureus</i> 3090(R)
Papiliocin	0.5	0.5	1	1	0.5	0.5	32	32
PapN	4	4	4	4	4	1	16	16
Melittin	4	4	4	4	4	2	2	2

implemented in Yasara software was used [21]. The LPS structure (PDB ID: 3FXI) was used as the receptor after adding hydrogens and performing quick minimization using the dreiding forcefield in Discovery Studio 4.1 (Accelrys Software Inc., USA) [22]. The PapN structure was used as a ligand. The N-terminus of papiliocin (PDB ID: 2LA2) was used to extract the PapN structure as the ligand with all the atoms fixed except the side chains of all residues in the structure. The entire LPS was set as the binding site using the AutoGrid algorithm, and AutoDock Vina was applied for the docking process. The ligand was given 125 docking runs and we retrieved the docked conformations through the “dock_play” protocol. The docking complex was subjected to 200 steps of steepest descent minimization using Discovery Studio 4.1 [22].

Statistical Analysis

For statistical analysis, at least three independent cell samples were included. We performed statistical tests using GraphPad InStat software (ver. 3.05; GraphPad Software, USA). We considered values as significant at $*p < 0.05$, $**p < 0.005$, and $***p < 0.001$.

Results

Antibacterial Activity

The antibacterial activity of PapN was measured by determining the MIC against eight drug-resistant bacteria. The MICs for parent papiliocin and melittin peptides were also determined. Similar to papiliocin, PapN showed potent antibacterial activity against all gram-negative bacteria tested (Table 1). The antibacterial activity of PapN against gram-negative bacteria was comparable to that of melittin, an AMP with high antibacterial activity. However, PapN and papiliocin showed a much less potent antibacterial activity against gram-positive bacteria compared with melittin. Our results showed that PapN has high selectivity against gram-negative bacteria. We further investigated the mechanism of this activity using fluorescence experiments.

Cytotoxicity against Mammalian Cells

The cytotoxicity of the peptide was tested against

NIH3T3 cells. The effects on cell survival were measured by the mitochondrial conversion of MTT to a colored formazan product, as shown in Fig. 1. PapN did not produce any cytotoxicity against NIH3T3 cells even at 100 μM , whereas the control peptide, melittin, was highly toxic even at concentrations below 10 μM . Papiliocin resulted in a survival rate of 62% at 100 μM .

Anti-Inflammatory Activities of PapN

LPS, commonly referred to as an endotoxin, is a major component of the outer membrane of gram-negative bacteria. Release of LPS induces NO production during septic shock. To test the potential anti-inflammatory activity of PapN and papiliocin, we measured their abilities to inhibit NO production in LPS-stimulated RAW264.7 cells. (Fig. 2A). Papiliocin exhibited much higher inhibition of NO production than PapN. However, treatment with PapN also showed a strong, dose-dependent inhibition of NO production. At 20 μM , PapN inhibited about 80% NO production compared with the control.

Inhibition of the production of the cytokine TNF- α in LPS-stimulated cells by PapN was investigated. PapN

Fig. 1. Dose-dependent response to the cytotoxic activity of PapN, papiliocin, and melittin in NIH3T3 cells.

Fig. 2. Anti-inflammatory activities of PapN.

(A) Inhibition of nitrite production by papiliocin and PapN in lipopolysaccharide (LPS)-stimulated (20 ng/ml) RAW264.7 cells. (B) Inhibition of TNF- α production by papiliocin and PapN. Peptide concentrations from 1 to 20 μ M were tested.

showed potent inhibition of TNF- α production (Fig. 2B), and at 20 μ M, PapN inhibited cytokine production by about 70%. Compared with PapN, papiliocin showed a much higher inhibition of NO production as well as TNF- α production. However, treatment with PapN also resulted in a strong, dose-dependent inhibition of NO and TNF- α production, proving that PapN is a potent anti-inflammatory peptide. These data imply that the C-terminal helix of papiliocin may play an improved effect with the N-terminal helix with respect to inhibiting NO and cytokine production, resulting in the high anti-inflammatory activity of papiliocin. Thus, the N-terminal helix PapN is uniquely important for the papiliocin's antibacterial activity, while also contributing to its anti-inflammatory properties.

Tryptophan Fluorescence Experiments

To investigate the interactions between tryptophan in the peptides and the gram-negative bacterial cell membrane, we monitored fluorescence emissions of Trp2 in papiliocin and PapN in SUVs composed of negatively charged

phospholipids or in LPS micelles. As the vesicles and LPS were dissolved in Tris and phosphate buffers, respectively, the results were compared separately with each buffer. Papiliocin and PapN showed a blue shift greater than 18 nm and 16 nm, respectively, in the presence of LPS. In comparison, they exhibited 11 nm and 12 nm blue shifts, respectively, in the presence of SUVs (Table 2). The large blue shift in LPS micelles suggested a close interaction with LPS in gram-negative bacteria.

To determine the extent to which Trp2 was buried in the gram-negative bacterial cell membrane, a fluorescence quenching experiment was performed with acrylamide as a quencher. This allowed the calculation of the Ksv for the quenching of Trp2 fluorescence by acrylamide in the presence of LPS (Table 3). The quenching of Trp2 fluorescence was equally effective with PapN as with papiliocin, suggesting that Trp2 of PapN and papiliocin was buried extensively within the negatively charged LPS, thus explaining the high inhibition of NO and cytokine production in LPS-stimulated RAW264.7 cells.

Table 2. Tryptophan fluorescence blue shift for papiliocin and PapN in various environments.

Peptides	λ_{max} (nm) in Phosphate buffer (pH 6.0)	λ_{max} (nm) in 8.0 μ M LPS	λ_{max} (nm) in Tris buffer	λ_{max} (nm) in EYPC/EYPG (7:3)
Papiliocin	353	335	351	340
PapN	354	338	351	339

Table 3. Quenching of tryptophan fluorescence in papiliocin and PapN by acrylamide.

Composition	Papiliocin		PapN	
	$\lambda_{\max}$	K_{sv}	$\lambda_{\max}$	K_{sv}
Phosphate buffer (pH 6.0)	352	30.46	354	29.25
LPS	335	7.39	338	7.92

CD Experiments

The secondary structure of PapN in LPS was investigated using CD spectroscopy. As shown in Fig. 3, in aqueous solution, PapN displayed a random structure. However, upon addition of LPS, the α -helical content of the peptide gradually increased in a dose-dependent manner with concentrations of up to 0.4 mg/ml LPS, implying that PapN acquired a more folded structure in the presence of LPS, which was used to mimic the gram-negative bacterial cell membrane environment.

Binding Model of LPS and PapN

Since CD data as well as fluorescence data proved that PapN interacts strongly with LPS and this interaction is necessary for its antibacterial and anti-inflammatory activities, we built a binding model of LPS and PapN. LPS consists of three main components: O-antigen, core oligosaccharide, and lipid A (Fig. 4A). The highly conserved lipid A portion with six lipid chains in LPS is key for inflammatory responses. Lipid A contains 3-hydroxy-tetradecanoic acid, myristic acid, lauric acid, phosphate ion, and 2-amino-2-deoxy- α -D-glucopyranose. Fig. 4B depicts papiliocin being composed of 37 amino acid residues with an amphipathic α -helical structure at the N-terminus and a hydrophobic C-terminal α -helix linked by a hinge region [6]. The docking pose of LPS and PapN showed that PapN interacts with LPS through the helix region of PapN (Figs. 4C and 4D). Fig. 4C shows the predicted binding pose, where LPS and PapN are displayed as spheres and surface, respectively. The hydrophobic surface and hydrophilic surface of PapN are shown as red and blue colors, respectively. In Fig. 4D, the PapN amphipathic helix can also be seen as a cartoon, and the residues interacting with LPS are shown as sticks. Our docking model suggested that LPS may exhibit several hydrophobic and electrostatic interactions with PapN (Figs. 4C and 4D). A previous time-killing analysis against *E. coli* as well as a transmembrane potential depolarization assay with *E. coli* revealed that papiliocin completely killed *E. coli* much faster and depolarized its membrane more effectively than papiliocin W2A or papiliocin F5A mutants [7]. Accordingly, Trp2 is

Fig. 3. CD spectra of PapN in the presence of 0–0.4 mg/ml LPS.

important for the antibacterial and anti-inflammatory activities of papiliocin [7]. Notably, in our binding model, Trp2 in PapN participated in hydrophobic interactions with the R2 lipid chain of LPS present in 3-hydroxy-tetradecanoic acid, akin to those of Phe5 with the R2' and R3 lipid chain of lauric acid and 3-hydroxy-tetradecanoic acid in lipid A (Fig. 4D). Furthermore, the binding model showed that the positively charged Arg1, Arg13, and Arg16 residues in the amphipathic N-terminal helix of papiliocin interacted electrostatically with the phosphate groups of lipid A in LPS (Fig. 4D). Positively charged side chains containing nitrogen atoms of Arg1, Arg13, and Arg16 shared electrostatic interactions with the phosphate of lipid A in LPS. The side-chain nitrogen atoms of Arg1 form hydrogen bond interactions with oxygen atoms in 2-amino-2-deoxy- α -D-glucopyranose and 3-hydroxy-tetradecanoic acid of lipid A. Likewise, the side-chain oxygen atom of Glu9 and the nitrogen atom in 2-amino-2-deoxy- α -D-glucopyranose of lipid A participated in hydrogen bond interactions.

Discussion

In NMR solution structure, papiliocin consists of an amphipathic α -helix from Lys3 to Lys21 linked by a hinge region to a hydrophobic α -helix from Ala25 to Val36 [6]. Papiliocin has a high net charge of +8 and contains seven Lys residues and three Arg residues. Notably, PapN alone possesses six Lys as well as three Arg residues, with a high net charge of +7. Given that PapN has a positively charged amphipathic α -helical structure, it retains the antibacterial and anti-inflammatory activities of papiliocin without cytotoxicity. This amphipathicity of PapN is critical for its

Fig. 4. Binding model of PapN to LPS.

(A) Three-dimensional structure of LPS shown as spheres. Fatty acid chains, phosphate groups, and sugar portions are shown as light pink, orange, and green colors, respectively. The region of lipid A, inner core, and outer core are shown. (B) Structure of papiliocin. The hydrophobic surface and hydrophilic surface of papiliocin are shown as red and blue colors, respectively. (C) Molecular docking pose of PapN and LPS. Spheres and surface are used to display LPS and PapN, respectively. The hydrophobic surface and hydrophilic surface of PapN are shown as red and blue colors, respectively. (D) Docking model of PapN bound to LPS. The PapN amphipathic helix is shown as a cartoon, and the residues interacting with LPS are shown as sticks. Hydrophobic and hydrophilic residues in the amphipathic helix of PapN are shown in red and blue colors, respectively.

antibacterial activity because the hydrophilic sector faces the negatively charged head groups of the phospholipid membrane, and the hydrophobic aromatic rings of Trp2 can penetrate into the acyl chains of hydrophobic lipids [8]. Furthermore, PapN exhibited potent antibacterial activities against various gram-negative multidrug-resistant bacteria, and significantly inhibited NO and cytokine production in LPS-stimulated RAW264.7 cells.

The main component of the gram-negative bacterial cell wall is LPS, which consists of a lipid A moiety and repeating polysaccharide moieties. LPS acts as a strong stimulator of macrophages and dendritic cells, which are part of the innate immunity of various organisms [11]. LPS covers the outer membrane of the gram-negative bacterial cell wall and triggers the secretion of inflammatory cytokines, resulting in septic shock and other immune diseases. The LPS-binding protein carries LPS and presents

it to the accessory protein called cluster of differentiation 14, which participates in TLR4 signaling after the active complex TLR4/MD-2/LPS is formed [23]. TLR4 signaling leads to proinflammatory cytokines that are important to activate potent immune responses [23]. However, uncontrolled inflammatory responses induced by LPS through TLR4 may lead to autoimmune diseases such as sepsis atherosclerosis, asthma, ischemia/reperfusion, and rheumatoid arthritis that can cause organ failure or even death, and thus anti-inflammatory activity is a vital process [24, 25]. Various biophysical experiments have investigated the interactions between AMPs and LPS [26, 27]. In this work, we used tryptophan fluorescence and CD spectroscopy to study the folding and the interactions between the Trp2 residue of PapN and LPS. The quenching of Trp2 fluorescence in PapN was effective in the presence of LPS micelles and was comparable to that of papiliocin. Trp2 at the N-terminus

appeared to be buried deeply inside the negatively charged LPS and, based on the large blue shift in the presence of LPS micelles, PapN was likely to interact tightly with LPS in gram-negative bacteria. Spectroscopic experiments involving CD also confirmed the gradual formation of an α -helical PapN structure upon addition of LPS, suggesting that this interaction resulted in potent anti-inflammatory activity of PapN.

Assuming that the anti-inflammatory activity of PapN might be related to the direct interaction between PapN and LPS, we used a docking study to investigate the binding model between LPS and PapN. Neutralization of LPS is a significant feature of AMPs' action against sepsis. AMPs such as lactoferrin, *Limulus* anti-lipopolysaccharide factor, cathelicidins, polymyxin B, and granulysins are promising peptides against sepsis owing to their direct interaction with LPS in gram-negative bacteria [15, 16]. Here, we showed that the positively charged Arg1, Arg13, and Arg16 residues of PapN interacted with the negatively charged phosphate of lipid A, and Trp2 and Phe5 in PapN engaged in hydrophobic interactions with the R2, R2'', and R3 acyl chain of lipid A. Docking models between LPS and AMPs such as cecropin P1, tachyplesin I, esculentin-1a(1-21)NH₂, and esculentin-1a(1-21)NH₂ 1c have been proposed [28–30].

High cationicity in the peptides that bind to LPS is required to neutralize the LPS. In our previous study, by checking the fluorescence intensity of FITC-conjugated LPS, we revealed the importance of electrostatic interactions between positively charged residues in PapN and LPS for LPS dissociation through mutation of the two key residues Trp2 and Phe5 in papiliocin [8]. Tachyplesin I (KWCVRV CYRGICYRRRCR-NH₂) is an AMP with six positively charged residues that can bind to LPS [30]. Moreover, NMR studies also prove that LPS interacts with Arg17, and phosphate groups of LPS may possibly bind to tachyplesin I. One docking model suggests that Arg14, Arg15, Arg17, and Lys1 participate in electrostatic interactions with LPS [30]. Similarly, Trp2 and Phe4 make hydrophobic interactions with the fatty acyl chains of LPS [30]. Cecropin P1 (SWL SKTAKKLENSAKKRISGIAIAIQGGPR) is another AMP that has been proven to bind with LPS [28]. The docking model shows that the residues Lys15 and Lys16 may interact with phosphate, and Ile22 and Ile26 may bind with lipid chains of LPS [28]. Esculentin-1a(1-21)NH₂ (GIFSKLAGK KIKNLLISGLKG-NH₂) is the other peptide that may bind to LPS through Lys5, Lys9, Lys12, and Lys20 [31]. Similarly, esculentin-1a(1-21)NH₂ 1c (GIFSKLAGKKIKN^dLLI^dSGLKG-NH₂) can bind with LPS through Lys5 and Lys10. NMR

studies suggest that these two peptides interact with the phosphate groups and fatty acyl chains of LPS through electrostatic and hydrophobic interactions, respectively [29]. Lauryl-LF11 peptide had antimicrobial activity against *Salmonella enterica* gram-negative bacteria, and electrostatic interactions were proven important for the peptide-LPS binding [8].

Similar to our docking pose, all those predicted poses displayed significant interactions between positively charged residues of peptide and negatively charged phosphate groups in the polar head of LPS. In addition, our model exhibited hydrophobic interactions between nonpolar fatty acid tails in LPS and hydrophobic residues of PapN. Hence, our molecular docking pose agrees with the other proposed binding modes between peptide and LPS.

In conclusion, PapN presents high antibacterial and anti-inflammatory activities, but without cytotoxicity. Importantly, PapN interacts strongly with LPS through Trp2 and Phe5 as well as positively charged Arg1, Arg13, and Arg16 in the amphipathic α -helical structure of PapN. These properties make PapN a potent peptide antibiotic suitable for treating the endotoxin shock and sepsis caused by gram-negative bacterial infections. Future in vivo studies will determine the potency of PapN for treating the septic shock caused by LPS of gram-negative bacteria and will confirm the current biophysical results as well as structure-function relationships of PapN.

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Conflict of Interest

The authors have no financial conflicts of interest to declare.

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