J. Biochem. 106, 663-668 (1989)

Antimicrobial Peptides, Isolated from Horseshoe Crab Hemocytes, Tachyplesin II, and Polyphemusins I and II: Chemical Structures and Biological Activity[1]

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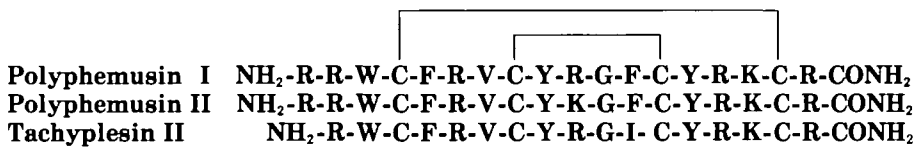
Received for publication, May 22, 1989

Tachyplesin is an antimicrobial peptide recently found in the acid extract of hemocytes from the Japanese horseshoe crab (*Tachypleus tridentatus*) [Nakamura, T. *et al.* (1988) *J. Biol. Chem.* 263, 16709-16713]. In our continuing studies on the peptide, we have found an isopeptide, tachyplesin II, and also polyphemusins I and II in hemocytes of the American horseshoe crab (*Limulus polyphemus*). The complete primary structures of these peptides, which are very similar to that of the previously isolated peptide, now named tachyplesin I, were determined to be as follows:



The isopeptide, tachyplesin II, consists of 17 residues with a COOH-terminal arginine α-amide. On the other hand, both polyphemusins I and II were found to contain 18 residues due to an additional Arg residue at the NH_2-terminal end as well as a COOH-terminal arginine α-amide. The disulfide linkages for polyphemusin I consisted of two bridges between Cys-4 and Cys-17 and between Cys-8 and Cys-13, which was identical to in the case of tachyplesin I. Moreover, all of these peptides inhibited the growth of not only Gram-negative and -positive bacteria but also fungi, such as *Candida albicans* M9. Furthermore, complex formation between these peptides and bacterial lipopolysaccharides was also observed in a double diffusion test. These results suggest that tachyplesins and polyphemusins are probably located in the hemocyte membrane, where they act on antimicrobial peptides as a self-defense mechanism in the horseshoe crab against invading microorganisms.

The circulating hemolymph in invertebrate animals is known to contain many biologically active substances, such as lectins, complement, clotting factors, and antimicrobial peptides, all of which contribute to a self-defense system in the animal kingdom against invading microorganisms (*1, 2*). In previous work, we reported that horseshoe crab hemocytes contain at least two polypeptide substances, named anti-lipopolysaccharide (LPS) factor (*3-6*) and tachyplesin (*7*), both of which neutralize a variety of LPS activities (*8-10*), and significantly inhibit the growth of Gram-negative and -positive bacteria (*4, 7*). One of these substances, tachyplesin, which is highly abundant in hemocyte debris of the Japanese horseshoe crab (*Tachypleus tridentatus*), has an unusual structure consisting of 17-amino acid residues with a COOH-terminal arginine α-amide. We report here studies on an isopeptide of tachyplesin and its analogues, named polyphemusins I and II, found in hemocyte debris from the American horseshoe crab (*Limulus polyphemus*). The chemical structures and biological functions of these highly active peptides suggest the existence of a new family of Arthropodous antibiotics, in addition to their precursor proteins, in horseshoe crab hemocytes.

[1] This work was supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science and Culture of Japan.

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Abbreviations: LPS, lipopolysaccharide; Boc, *N-tert*-butoxycarbonyl; pNA, *p*-nitroanilide; HPLC, high performance liquid chromatography; FAB, fast atom bombardment; Pe, *S*-pyridylethyl; Ae, *S*-aminoethyl; PTH, phenylthiohydantoin.

MATERIALS AND METHODS

Materials—*Tachypleus tridentatus* hemocytes were collected by means of previously described methods (*4*). *L. polyphemus* hemocytes and *N-tert*-butoxycarbonyl-Val-

Pro-Arg-*p*-nitroanilide were kind gifts from Mr. H. Nakajima (Taiyo Fishery, Tokyo) and Mr. S. Tanaka (Seikagaku Kogyou, Tokyo), respectively. An LPS-sensitive serine protease zymogen, factor C, from *Tachypleus* hemocytes was highly purified by methods described previously (*8*). Synthetic tachyplesins I and II, and polyphemusin I were kindly supplied by Dr. N. Fujii (Faculty of Pharmaceutical Sciences, Kyoto University). Trypsin, treated with *N*-tosyl-L-phenylalanyl chloromethyl ketone, was obtained from Worthington Biochemical, Freehold, N.J. Lysylendopeptidase from *Achromobacter lyticus* M497-1 and *S*-(2-aminoethyl)-L-cysteine were obtained from Wako Pure Chemical Industries, Tokyo. LPS purified from *Escherichia coli* 0111:B4 and arginine α-amide were purchased from Sigma Chemical, St. Louis, Mo. Arginine α-amide was also a kind gift from Dr. H. Hirata (Kowa, Tokyo). Sephadex G-50 was from Pharmacia Fine Chemicals, Uppsala. Cosmosil 5C18 and Chemcosorb 7 ODS-H were obtained from Nacalai Tesque, Kyoto, and Chemco Scientific, Tokyo, respectively. All other chemicals were of analytical grade.

Purification of Tachyplesin II and Polyphemusins—A cationic peptide from the hemocytes was extracted with 20 mM HCl and then applied to a Sephadex G-50 column according to the previously described method (*7*). The fractions containing tachyplesins or polyphemusins were concentrated by lyophilization and then dissolved in 10 ml of 20 mM HCl. The samples were injected, respectively, onto a Cosmosil 5C18 (8×250 mm) column. Elution was performed with a linear gradient (21.6-29.6%) of acetonitrile containing 0.1% trifluoroacetic acid for 40 min at the flow rate of 1.0 ml/min.

Assay for Tachyplesins and Polyphemusins—These cationic peptides were assayed as to their inhibitory effect on LPS-mediated activation of factor C (*7, 8*). During HPLC purification, 20 μl of each peak material was assayed. One unit of the tachyplesin and polyphemusin activities was defined as the amount that inhibited 50% of the factor C activation mediated by 0.2 μg of LPS (*7*).

Reduction and S-Alkylation—The tachyplesin and polyphemusin peptides were reduced and *S*-pyridylethylated or *S*-aminoethylated for sequence determination or identification of the COOH-terminal amino acid residue (*5, 11*).

Amino Acid and Sequence Analysis—Amino acid analysis of tachyplesin peptides was performed as described previously (*7*), using a Hitachi L-8500 automatic analyzer. The sequence analysis of *S*-pyridylethylated or *S*-aminoethylated samples was carried out with an Applied Biosystems 477A gas-phase sequencer and a 120A PTH-analyzer on-line system.

Determination of the COOH-Terminal Residues of Tachyplesin II and Polyphemusins I and II—In order to identify the COOH-terminal end, an *S*-aminoethylated sample was digested with lysylendopeptidase and the resulting free arginine α-amide was analyzed with a PICO·TAG amino acid analysis system (Waters, Millipore, Milford, Mass.), as described previously (*7*). The COOH-terminal residue of polyphemusin I was also identified by fast atom bombardment (FAB) mass spectrometry. Native and Ae-polyphemusins I were submitted to mass measurement as described elsewhere (*12*).

Assignments of Disulfide Bridges in Polyphemusin I—The positions of disulfide linkages in polyphemusin I were determined by the previously described method (*7*), except that a Chemcosorb 7 ODS-H (2.1×150 mm) column was employed for separation of the disulfide-containing peptides.

Double Diffusion Test—Complex formation between native or synthetic tachyplesin peptides and LPS was analyzed by a double diffusion method on a 1% agarose gel plate (*7, 13*). The double diffusion was carried out in a moist chamber at room temperature for 2 d. The precipitin lines formed between samples and LPS were stained with Coomassie Brilliant Blue R-250 after drying the gel plate with filter paper (*8*).

Antimicrobial Activity—The following strains were used for determination of the antimicrobial activity; *Salmonella typhimurium* LT2 and 1102, *Escherichia coli* K12, *Staphylococcus aureus* 209P and ATCC 25923, *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Salmonella minnesota* 1114W and R595, *Candida albicans* M9, and *Cryptococcus neoformans* IMF 40040. Heart infusion broth (Difco Laboratories, Detroit) was used for subculturing of the bacteria. For growth inhibition tests on the bacteria, a synthetic Jarvis's medium (*14*) (abbreviated as JY medium) supplemented with 2 mg/ml of yeast extract was employed (*3*), and the fungi were grown in Sabouraud broth at 30°C for 2 d, minimal inhibitory concentrations (MIC) being determined, as described previously (*7*). The MIC was expressed as the lowest final concentration at which no growth was observed.

RESULTS

Isolation of Tachyplesin II and Polyphemusins I and II—In the previous study (*7*), tachyplesin was finally purified by ion exchange chromatography on a CM-Sepharose column, after gel filtration of the acid extract of the *Tachypleus* hemocyte debris on a Sephadex G-50 column. When reversed-phase HPLC instead of an ion exchange column was employed for separation of the tachyplesin fraction obtained on gel filtration, we found two peptides, both of showed tachyplesin activity, as shown in Fig. 1S. The earlier peak, eluted at 28.8 min, was named tachy-

TABLE I. **Amino acid compositions of *S*-aminoethylated tachyplesin II and polyphemusins I and II, and the observed mass values for the native materials.**

Amino acid	Tachyplesin II	Polyphemusin I	Polyphemusin II
	(Residues per molecule)		
Gly	1.2 (1)[a]	1.2 (1)	1.2 (1)
Arg	5.0 (5)	5.8 (6)	4.8 (5)
Tyr	2.1 (2)	1.9 (2)	1.8 (2)
Val	1.0 (1)	1.1 (1)	1.0 (1)
Ae-Cys[b]	4.2 (4)	3.3 (4)	3.1 (4)
Ile	1.0 (1)		
Phe	1.1 (1)	2.0 (2)	2.0 (2)
Trp[c]	1.2 (1)	0.9 (1)	0.8 (1)
Lys	1.0 (1)	1.1 (1)	2.0 (2)
Total	17	18	18
$M+H^+$	2,263.2 (2,263.1)[d]	2,452.9 (2,453.2)	2,452.0 (2,452.2)

[a]Values in parentheses are taken from the sequence data. [b]*S*-aminoethylcysteine. [c]Obtained on 20 h hydrolysis with 3 M mercaptoethanesulfonic acid. [d]Values in parentheses are theoretical masses calculated from the sequences with arginine α-amide at the COOH-terminal ends.

plesin II and the later peak, which eluted at 30.0 min, was named tachyplesin I, which was also found to be identical to the tachyplesin previously isolated (7), based on its amino acid composition and sequence. In summary, the acid extract of the *Tachypleus* hemocyte debris was found to contain two isopeptides with tachyplesin activity.

On the other hand, the acid extract prepared from American horseshoes crab (*Limulus polyphemus*) hemocyte debris was first fractionated on a Sephadex G-50 column (Fig. 2S). Polyphemusin activity was found in a low molecular weight fraction, which was further purified on a Cosmosil 5C18 column by reversed-phase HPLC. As shown in Fig. 3S, two major peaks were separated which showed polyphemusin activity. The yields of polyphemusins I and II were about 2.2 mg and 0.7 mg, respectively, from 99.5 g of hemocytes (wet weight). These polyphemusins thus obtained were subjected to structural studies.

Amino Acid Sequences of Tachyplesin II and Polyphemusins I and II—Table I shows the amino acid compositions of tachyplesin II and polyphemusins I and II. Tachyplesin II was found to consists of 17 residues, while both polyphemusins I and II contained 18 residues. There were no acidic amino acids or hexosamines, which was similar to in the case of tachyplesin I. The amino acid sequences were elucidated by automated Edman degradation using *S*-pyridylethylated or *S*-aminoethylated derivatives. The results are shown in Table II. Although a free PTH-arginine was detected at the COOH-terminal ends of these peptides using a gas-phase sequencer, this must be derived from a COOH-terminal arginine α-amide resulting from a deamidation reaction under the acidic conditions. These sequence analyses indicated that tachyplesin II has an NH_2-terminal arginine, which is different from in the case of tachyplesin I, which has an NH_2-terminal lysine (7). Furthermore, these peptides differd in an additional single amino acid at position 15 from the NH_2-terminus; an arginine in tachyplesin I and a lysine in tachyplesin II.

On the other hand, a difference between polyphemusins I

TABLE II. **Amino acid sequences of *S*-pyridylethylated tachyplesin II and polyphemusin I and *S*-aminoethylated polyphemusin II[a].**

Cycle No.	Tachyplesin II		Polyphemusin I		Polyphemusin II	
	PTH amino acid	Yield (pmol)	PTH amino acid	Yield (pmol)	PTH amino acid	Yield (pmol)
1	Arg	1,575	Arg	214	Arg	606
2	Trp	542	Arg	198	Arg	631
3	Pe-Cys	n.q.[b]	Trp	204	Trp	354
4	Phe	674	Pe-Cys	n.q.	Ae-Cys	n.q.
5	Arg	2,241	Phe	581	Phe	493
6	Val	588	Arg	318	Arg	651
7	Pe-Cys	n.q.	Val	439	Val	477
8	Tyr	633	Pe-Cys	n.q.	Ae-Cys	n.q.
9	Arg	2,244	Tyr	148	Tyr	371
10	Gly	469	Arg	249	Lys	339
11	Ile	561	Gly	329	Gly	402
12	Pe-Cys	n.q.	Phe	278	Phe	412
13	Tyr	599	Pe-Cys	n.q.	Ae-Cys	n.q.
14	Arg	1,996	Tyr	370	Tyr	295
15	Lys	1,494	Arg	546	Arg	352
16	Pe-Cys	n.q.	Lys	889	Lys	335
17	Arg	1,457	Pe-Cys	n.q.	Ae-Cys	n.q.
18			Arg	696	Arg	505

[a]The COOH-terminal Arg α-amide was identified as PTH-Arg, with a gas-phase sequencer, due to the deamidation reaction under acidic conditions. [b]n.q., not quantitated.

TABLE III. **Antimicrobial activity of native and synthetic tachyplesins and polyphemusins.**

Strain	Minimal inhibitory concentration (μg/ml)						
	Tachyplesin I		Tachyplesin II		Polyphemusin I		Polyphemusin II
	Native	Synthetic	Native	Synthetic	Native	Synthetic	Native
Gram (−)							
Salmonella typhimurium LT2	3.1	1.6-3.1	3.1	1.6-3.1	3.1	3.1-6.3	6.3
Salmonella typhimurium 1102	0.8-1.6	0.8-1.6	1.6	1.6-3.1	3.1	3.1	3.1
Escherichia coli K12	1.6-3.1	1.6-3.1	3.1	1.6-3.1	6.3	6.3	12.5
Salmonella minnesota 1114W	3.1	6.3	3.1	6.3	6.3	12.5	12.5
Salmonella minnesota R595	1.6	1.6	1.6	1.6	3.1	3.1	3.1
Pseudomonas aeruginosa	12.5	—	—	—	—	—	—
Gram (+)							
Staphylococcus aureus 209P	3.1	3.1	1.6-3.1	6.3	6.3	6.3	6.3
Staphylococcus aureus ATCC 25923	6.3	12.5	6.3	12.5	6.3	6.3	12.5
Bacillus subtilis	3.13	—	—	—	—	—	—
Fungus							
Candida albicans M9	3.1	3.1	3.1	3.1	6.3	6.3	6.3
Cryptococcus neoformans IMF40040	1.56	—	—	—	—	—	—

and II, consisting of 18 residues, was only found at position 10, with an arginine in the former and a lysine in the latter.

Identification of the COOH-Terminal Ends of Tachyplesin II and Polyphemusins I and II—In order to identify the COOH-terminal ends of these peptides, *S*-aminoethylated tachyplesin II (22.5 nmol) and polyphemusins I (27.4 nmol) and II (6.8 nmol) were first digested with lysylendopeptidase, which specifically hydrolyzes peptide bonds on the carboxyl side of lysine and *S*-aminoethylcysteine residues; the digests were then coupled with phenyl*iso*thiocyanate. The products were analyzed by PICO·TAG amino acid analysis without acid hydrolysis (*7*). In addition to free *S*-aminoethylcysteine (as the phenylthiocarbamoyl-derivative) released from the COOH-terminal region of these peptides, a peak with the same retention time as that of authentic arginine α-amide was found, as shown in Fig. 4S. The molar ratios of *S*-aminoethylcysteine/arginine α-amide in tachyplesin II and polyphemusins I and II were 0.96, 0.73, and 0.56, respectively. The results indicated that all of the COOH-terminal residues of these peptides were not arginine but arginine α-amide.

The presence of an arginine α-amide in these three peptides was also confirmed by FAB mass spectrometric analysis. Figure 5S shows FAB mass spectra of native and *S*-aminoethylated polyphemusin I, in which the isotopic molecular ion distributions were quite similar to that observed for tachyplesin I (*7*). Both of the observed mass values of 2,452.9 and 2,629.0 (protonated form) were nearly identical to the theoretical values calculated from the sequence with arginine α-amide at the COOH-terminal end. Native polyphemusin II and tachyplesin II were also examined. Their mass values were consistent with the theoretical values, as shown in Table I. These findings indicate that all of these three peptides have a COOH-terminal amide group.

Assignment of Disulfide Linkages in Polyphemusin I—No *S*-aminoethylcysteine was found on amino acid analysis after treatment of native polyphemusin with 4-vinylpyridine in the absence of dithiothreitol, indicating the presence of two disulfide linkages in the peptide. The disulfide linkages of polyphemusin I were assigned on the basis of the amino acid compositions of tryptic peptides derived from native polyphemusin I. Figure 6S shows the elution profile of a tryptic digest on a Chemcosorb 7 ODS-H column. The amino acid compositions of the peptides are listed in Table IS. Peptide T1 was a pentapeptide (Arg-2 to Arg-6) linked with a tripeptide (Lys-16 to Arg α-amide-18). Peptide T2 was a tetrapeptide (Trp-3 to Arg-6) linked with a tripeptide (Lys-16 to Arg α-amide-18). Peptide T3 was a nonapeptide (Val-7 to Arg-15) containing a disulfide linkage. These results indicated the presence of two disulfide linkages between Cys-4 and Cys-17 and between Cys-8 and Cys-13.

Complex Formation between Tachyplesin II or Polyphemusin I or II and LPS—Complex formation between the native peptides (30 μg) and LPS (20 μg) was analyzed by a double diffusion test on an agarose gel plate. As shown in Fig. 7S, polyphemusins I and II formed a precipitin line with LPS, respectively, suggesting the formation of a high molecular weight complex. The same results were obtained with chemically synthesized tachyplesins I and II and polyphemusin I (*15*), a precipitin line also being formed between these peptides and LPS in the double diffusion test.

Antimicrobial Activity of Native and Synthetic Peptides—The minimal inhibitory concentrations of tachyplesins I and II and polyphemusins I and II for various bacterial strains were determined by the microplate culture method (*7*). The chemically synthesized peptides were also tested for comparison. As summarized in Table III, native tachyplesin II and polyphemusins I and II displayed potent antimicrobial activity toward several microbial strains. Tachyplesin II showed almost the same potency and inhibitory effect spectrum as those of tachyplesin I against Gram-negative (*Salmonella* and *Escherichia* strains) and Gram-positive bacteria, such as *Staphylococcus* species. In general, polyphemusins I and II showed the same or somewhat lower potency than the tachyplesins. Furthermore, the growth of fungi, such as *Candida albicans* M9 and *Cryptococcus neoformans* IMF 40040, was also strongly inhibitied by tachyplesin peptides. On the other hand, synthetic tachyplesins I and II and polyphemusin I showed the same or a rather strong inhibitory effect on the growth of microorganisms, as compared with the native preparations. These antimicrobial potencies of native and synthetic peptides were comparable to that of anti-LPS factor reported previously (*4*).

DISCUSSION

Based on the above results, the chemical structures of antimicrobial peptides isolated from hemocytes of *Tachypleus tridentatus* and *Limulus polyphemus* were unambiguously established, as summarized in Fig. 1. Tachyplesin I consists of a total of 17 amino acid residues with a COOH-terminal arginine α-amide (*7*). Polyphemusins I and II were found for the first time in American horseshoe

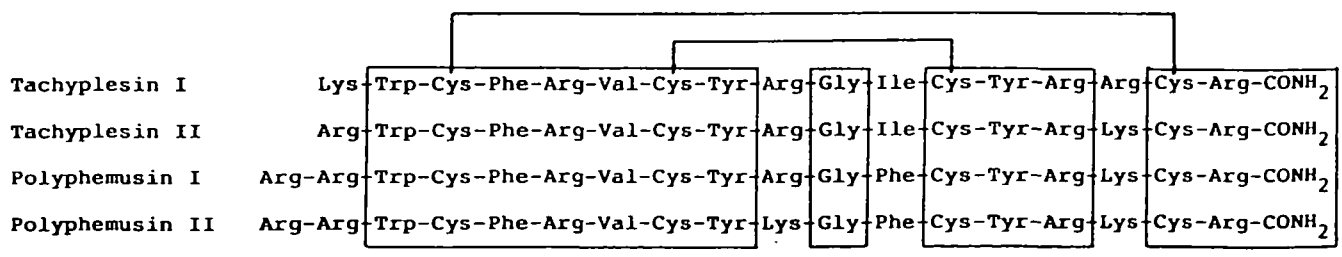

Fig. 1. **Amino acid sequences of tachyplesins I and II and polyphemusins I and II.** Residues identical in all peptides are boxed. Disulfide linkages are shown as solid lines.

crab hemocytes. They consist of 18 residues with an additional Arg at the NH_2-terminus, the only structural difference between being the residue, Arg and Lys, respectively, at position 10. The locations of the two disulfide linkages in polyphemusin I are identical to those in the case of tachyplesin I (*7*). This suggests strongly that those in tachyplesin II and polyphemusin II are also similar to those in tachyplesin I, although their full assignments have not been made to date. Moreover, all of these structures have recently been confirmed by comparing them with those of synthetic analogs on HPLC (*15*).

It is of interest that these new peptides, as well as tachyplesin I (*7*), show a characteristic structure with three tandem repeats of a tetrapeptide sequence; namely, hydrophobic amino acid (*i.e.*, Trp/Val/Ile/Phe)-Cys-hydrophobic amino acid (*i.e.*, Phe/Tyr)-Arg(Lys), indicating that an amphipathic nature is closely associated with their biological activity. ^{1}H-NMR determination of the solution structure of tachyplesin is now in progress.

As reported previously (*7*), tachyplesin I displays antimicrobial activity toward both Gram-negative and -positive bacteria. The analogous peptides described here have almost the same potency as that of tachyplesin I, as judged on the basis of the results with the microplate culture method (Table III). Interestingly, all of these peptides, including tachyplesin I, are effective against the growth of *Candida albicans* M9. In the presence of tachyplesin I or II at 3 μg/ml, this fungus irreversibly lost viability (data not shown). These results suggest that the tachyplesin family found in hemocyte debris function as antimicrobial peptides for the biological defense of the horseshoe crab against invading microorganisms.

Naturally occurring peptides containing an arginine α-amide at the COOH-terminus are known to exist in scorpion polypeptide toxin (*16*) and sarcotoxins from *Sarcophaga peregrina*. The latter exists in the pre-pro form with a COOH-terminal glycine residue, as judged from the cDNA sequence cloned for sarcotoxin (*17, 18*). Studies are currently in progress to identify the precursor protein and to determine the localization of tachyplesins in horseshoe crab hemocytes.

We wish to express our thanks to Ms. Satsuki Kajiyama and Yuko Nishina for the amino acid analysis, Dr. Lawrence J. Berliner for his comments on the English manuscript, and also Miss Nobuko Ueno for her expert secretarial assistance.

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Supplemental Materials

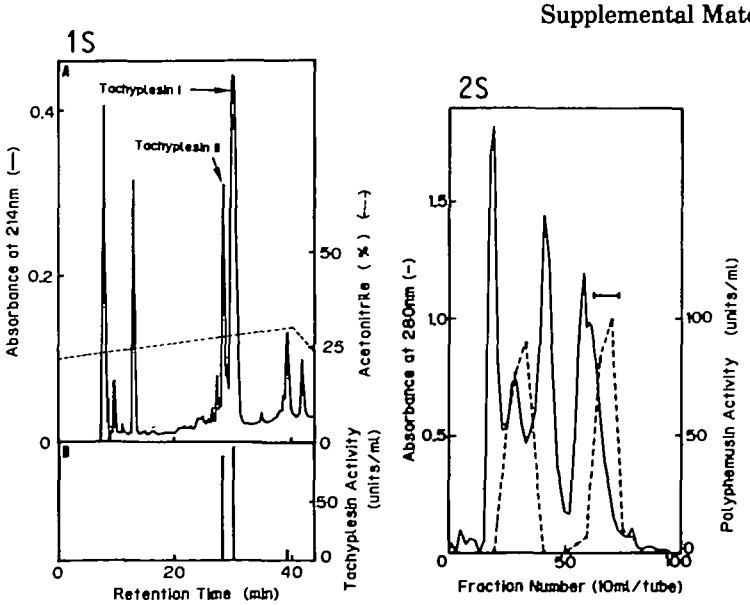


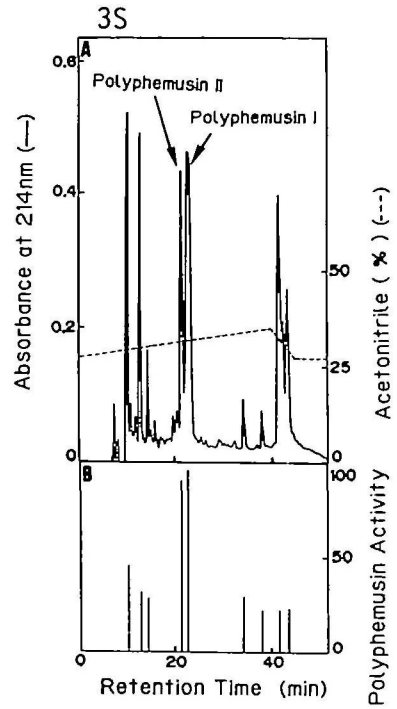
TABLE IS. Amino acid compositions of tryptic peptides derived from polyphemusin I.

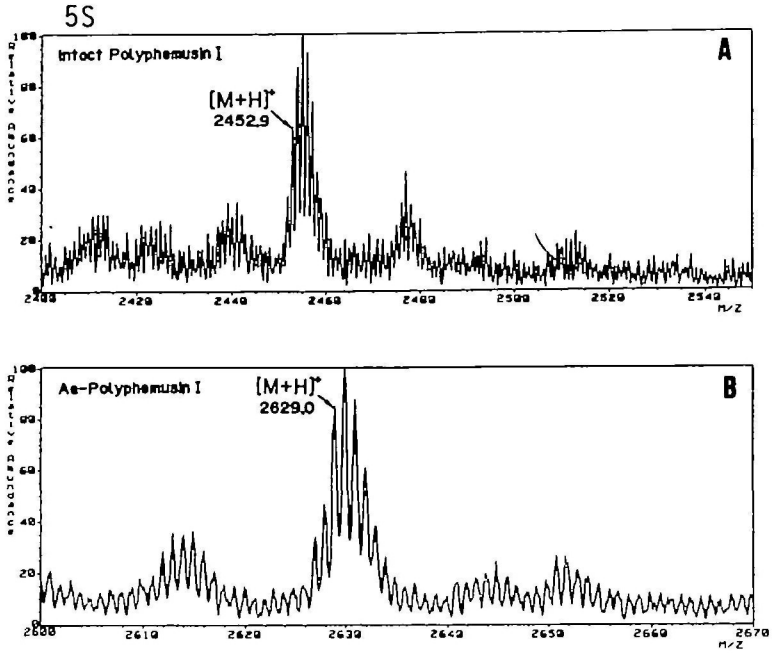
Amino acid	T1	T2	T3	T4	T5
	Residues per molecule				
Gly			1.1 (1)	1.4 (1)	1.4 (1)
Arg	3.2 (3)	1.8 (2)	2.1 (2)	5.9 (6)	4.3 (4)
Tyr			2.0 (2)	2.0 (2)	1.8 (2)
Val			1.0 (1)	1.1 (1)	0.9 (1)
Cys-SO_3H	1.7 (2)	1.9 (2)	1.6 (2)	2.8 (4)	2.7 (4)
Phe	1.0 (1)	1.0 (1)	1.0 (1)	2.0 (2)	1.7 (2)
Trp[a]	0.8 (1)	0.9 (1)		1.1 (1)	0.9 (1)
Lys	0.7 (1)	0.4 (1)		1.2 (1)	1.0 (1)
Total	8	7	9	18	16
Position	(2-6) (16-18)	(3-6) (16-18)	(7-15)	(1-18)	(3-18)

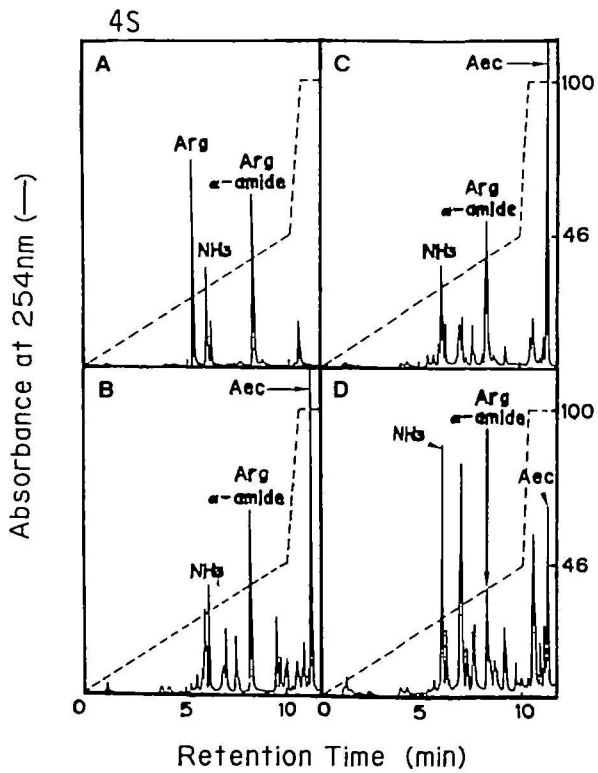
a: Obtained on 20h hydrolysis with 3M mercaptoethanesulfonic acid.

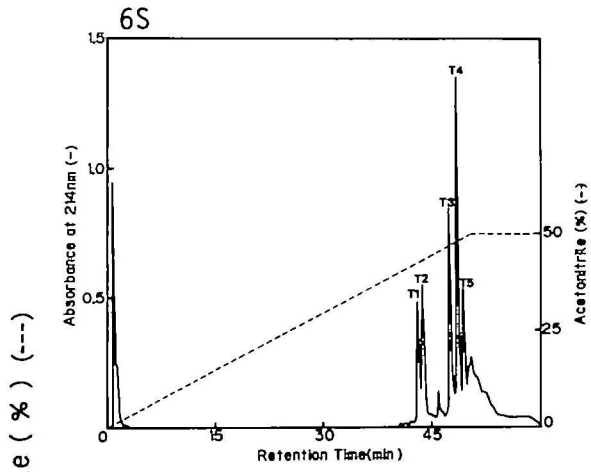
Fig. 1S. Separation of tachyplesins I and II by HPLC. The tachyplesin sample (*7*), purified by gel filtration on a Sephadex G-50 column, was applied to a Cosmosil 5C18 (8 x 250 mm) column at the flow rate of 1.0 ml/min (A). The tachyplesin activity of each peak is shown in (B). For other details, see "Methods".

Fig. 2S. Gel filtration of an acid-extract obtained from American horseshoe crab hemocyte debris on a Sephadex G-50 column. The absorbance at 280 nm (——) and polyphemusin activity (----) are shown. On amino acid analysis, the earlier peak material, eluted at fractions 20 - 40, was identified as coagulin, which also shows LPS-neutralizing activity (data not shown). The fraction indicated by the solid bar was collected. Details of the procedure are given under "Materials and Methods".









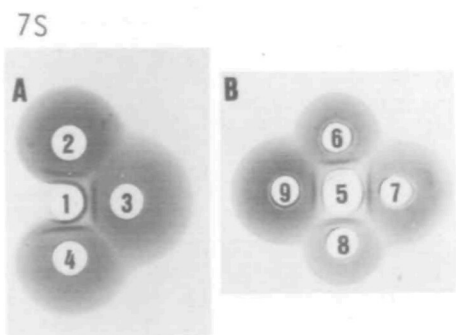


Fig. 3S. Separation of polyphemusins I and II by HPLC. The polyphemusin fraction (400 µl) (Fig. 2S) was applied to a Cosmosil 5C18 (8 x 250 mm) column at the flow rate of 1.0 ml/min (A). The polyphemusin activity of each peak is shown in (B).

Fig. 4S. Identification of the COOH-terminal amino acid residues of tachyplesin II, and polyphemusins I and II. The lysyl endopeptidase digests from S-aminoethylated peptides were coupled with phenylisothiocyanate and the resulting phenylisothiocarbamoyl derivative was separated by reversed-phase HPLC. A, synthetic arginine α-amide and arginine; B, C and D, lysyl endopeptidase digests obtained from S-aminoethylated tachyplesin II, and polyphemusin I and polyphemusin II. Aec, phenylisothiocarbamoyl-S-aminoethylcysteine.

Fig. 5S. Fast atom bombardment mass spectra of native polyphemusin I (A) and S-aminoethylated polyphemusin I (B). M denotes molecular weight.

Fig. 6S. Separation of tryptic peptides derived from native polyphemusin I by HPLC. The tryptic digest of native polyphemusin I was applied to a Chemcosorb 7 ODS-H column and eluted at the flow rate of 0.5 ml/min with a linear gradient of acetonitrile containing 0.1 % trifluoroacetic acid.

Fig. 7S. Analysis of the interaction of synthetic (A) and native (B) tachyplesins and polyphemusins with LPS. The complex formation between peptides (30 µg) and LPS (20 µg) was analyzed in 1 % agarose gel containing 50 mM Tris-HCl buffer (pH 9.0). 1, 5, LPS; 2, synthetic tachyplesin I; 3, synthetic tachyplesin II; 4, synthetic polyphemusin I; 6, native tachyplesin I; 7, native tachyplesin II; 8, native polyphemusin I; 9, native polyphemusin II.