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Bioenvironmental Interaction of Toxic Peptide from Hornet Venom with Phospholipid

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Toxic peptides from hornet venom, mastoparan and mastoparan-B were synthesized using the solid phase peptide synthesis method and investigated the interaction of them with phospholipid bilayer, antibacterial activity, and hemolytic activity. Both toxic peptides could induce dye release at a low concentration in neutral liposome. The binding affinity of mastoparan-B for neutral liposome was smaller than that for acidic one. Mastoparan and mastoparan-B had strong antibacterial activity for gram-positive bacteria, but weak or potent activity for gram-negative ones, respectively. Mastoparan and mastoparan-B lysed erythrocyte very little up to 5 μM.

Key words : toxic peptide, hornet venom, phospholipid

1. Introduction

A major component of the wasp venom, mastoparan, is a basic amphiphilic toxic peptide composed of 14 amino acid residues(Nakajima et al, 1986). Mastoparan toxin possesses various biological activities such as erythrocyte lysis, mast cell degradation(Hirai et al, 1983), histamine release, activation of phospholipase and binding to calmodulin(Malencik et al, 1983). It causes the secretion of serotonin from platelet, catecholamines from chromaffin cell, and prolactin from the anterior pituitary. Mastoparan is also turned out to enhance the permeability of artificial membrane and biomembrane(Katsu et al, 1990). Argiolas and Pisano(1980) have shown that mastoparan could bind to liposome containing phosphatidylcholine and that this binding correlated with the stimulation of the hydrolysis of phosphatidylcholine by phospholipase. Mastoparan displays a novel mode of toxicity by acting directly on guanosine triphosphate-binding regulatory protein to mimic the role normally played by agonist-liganded receptors. Structure-activity relationship studies with the use of various natural and synthetic compounds have shown that the amphiphilic structure with cationic amino acid residues on one side and hydrophobic residues on the other side was crucial in showing above biological activity (Higashijima et al, 1990). Recently a new kind of mastoparan, mastoparan-B has been isolated from the hornet venom(Ho et al, 1991). This toxic peptide caused the liberation of histamine from rat peritoneal mast cell and possessed stronger hemolytic activity than mastoparan. Mastoparan-B has been shown to elicit cardiovascular depressor(Ho et al, 1994) and to inhibit the growth of bacteria. The

more hydrophilic surface on the mastoparan molecule might lead to the change in its interaction with membrane, resulting in the alteration of its biological activity.

Study on the interaction of mastoparan and mastoparan-B with liposome has not yet been the subject of any other reports. Thus in order to obtain further information for the relationship between the hydrophilic side in the amphiphilic structure and the activity of toxic peptide, we determined the leakage and binding ability in neutral and acidic liposome, the antibacterial activity, and the hemolytic activity of toxic peptide.

2. Materials and Method

2.1 Synthesis and purification of toxic peptide from hornet venom

Toxic peptide, mastoparan(amino acid sequence was Ile-Asn-Leu- Lys- Ala- Leu-Ala-Ala-Leu-Ala-Lys-Lys-Ile-Leu-NH_2) and mastoparan-B(amino acid sequence was Ile-Lys-Leu-Lys-Ser-Ile-Val-Ser-Trp-Ala-Lys-Lys-Val- Leu- NH_2) were synthesized according to the Fmoc-chemical procedure starting from Fmoc-NHSAL resin using solid phase peptide synthesis method. After trifluoroacetic acid(TFA) / thioanisole/ ethanedithiol/m-cresol (20:5: 3:1, v/v) treatment for 2 h to eliminate all the protecting groups and resin, the crude peptide was dissolved in 10% acetic acid and purified by Sephadex G-25 with 10% acetic acid, and then by HPLC (Waters 600 system with ODS column, 3. 9 x 300 mm) with a gradient system of water-acetonitrile containing 0.1% TFA. Amino acid analysis was carried out using HPLC amino acid system equipped with FP-210 spectrofluorometer after hydrolysis of the peptide by 6.0 M HCl in sealed tube at 110℃ for 24 h. Molecular weight was determined by Fast Atom Bombardment mass spectra(FAB-MS). Peptide concentrations for mastoparan and mastoparan-B were determined from UV-absorbance of Trp in 8M urea.

2.2 Preparation of liposome

Unilamellar vesicles were prepared with a lipid composed of dipalmitoyl-d, l-phosphatidyl choline(DPPC), dipalmitoyl-d, l-phosphatidyl choline-dipalmitoyl-d, l-phosphatidyl glycerol(DPPC-DPPG, 3:1) as neutral and acidic vesicle, respectively. Phospholipid (20mg, 25mmol) was dissolved in $CHCl_3$(1 mL) and dried in a glass tube. The dried lipid was hydrated with 2 mL of 5mM 4-(2- hydroxyethyl)-1-piperazineethanesulfonic acid(HEPES) buffer (pH 7.4) with mixing at 50℃ for 30 min and diluted to 25 mL with the same buffer (lipid concentration, 1.0 mM). The unilamellar vesicles trapping carboxyfluorescein were prepared by the same method as described above except that the dried lipid (20 mg, 25 mmol) was hydrated in 2 mL of 0.1M NaCl/5 mM buffer(pH 7.4) containing 100 mM carboxyfluorescein. After sonication, the mixture of uni- and multilamellar vesicles trappimg carboxyfluorescein was subjected to gel filtration through Sephadex 4B column (1 x 20 cm) in 0.1M NaCl/5 mM buffer(pH 7.4). 2 mL fractions were collected and the fraction which was just before the non-encapsulated dye-elution was collected for the carboxyfluorescein release measurement. The lipid concentration of the fraction was 2.8 mM.

2.3 Leakage of carboxyfluorescein from liposome by toxic peptide

Leakage of liposome was determined with the fluorescein dye-release experiment from liposome by the procedure of Weinstein et al.(1977) with a minor modification(Suenaga et al, 1988). Lipo-

some containing 100 mM carboxyfluorescein was prepared by sonication as described above. A liposome fraction obtained by passing through Sepharose 4B was added to 2 mL of 5 mM HEPES buffer(pH 7.4) to give a final concentration of the peptide.

2.4 Spectroscopic titration of toxic peptide with liposome

Spectroscopic titration of peptide with liposome was performed as the Surewicz and Epand method(1984). Appropriate aliquot of DPPC or DPPC-DPPG(3:1) liposome(1.0 mM) was successively added to toxic peptide solution(2.0 mL) in HEPES buffer. After the addition of liposome, the mixture was kept at 25℃ for 10 min. From fluorescence spectra excited at 280 nm, the change of the maximum emission wavelength in fluorescence of Trp was used to determine the affinity parameter Kd/n, where Kd represented the dissociation constant of lipid-peptide complex and n the number of binding site per lipid.

2.5 Antibacterial activity of toxic peptide

Mastoparan and mastoparan-B were in vitro evaluated for antibacterial activity by determining the minimum inhibitory concentration(MIC) with serial 2-fold dilution method for gram-positive and -negative bacterial cells(Victor, 1988). Inoculum was standardized to equal a 0.5 McFarland turbidity standard and then further diluted 1:200 to achieve 10^5 cells/mL. Begining with 100 μg/mL, diluted it 2-fold serially with Muller Hinton Broth and incubated overnight at 37℃. MIC was determined by the difference of transmittance between control and each toxic peptide using spectrophotometer.

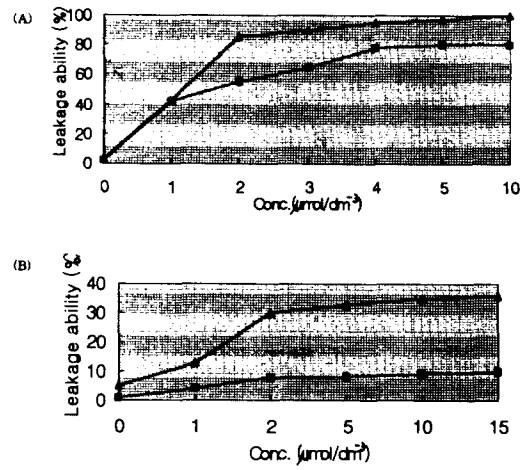

Fig. 1. Leakage profile of carboxyfluorescein from DPPC (A) and DPPC-DPPG (B) liposome by mastoparan (▲) and mastoparan-B (■).

2.6 Hemolytic assay of toxic peptide

The buffy coat was removed by centrifugation of fresh human blood and the erythrocyte was washed three times with isotonic saline and stored at 4℃. It was incubated with toxic peptide at 37℃ in 10mM phosphate buffer containing 150mM NaCl buffered saline and then centrifuged. The absorbance of the supernatant was measured at 542 nm. The absorption degree of the supernatant obtained by the treatment of erythrocyte with 1% triton X-100 was taken as 100%.

3. Results and Discussion

3.1 Leakage of liposome

In order to study the interaction of toxic peptide with model membrane, the ability of membrane perturbation was examined by measuring its effect of the release of carboxyfluorescein from phospholipid liposome. Profiles of the dye-leakage from DPPC and DPPC-DPPG(3:1) liposomes caused by toxic peptide were shown in Fig. 1. Toxic peptide could induce a complete

Table 1. Antibacterial activity of mastoparan and mastoparan-B

Bacteria	Mininum inhibitory concentration (μg/mL)	
	mastoparan	mastoparan-B
Bacilus subtilus PCI 219	3.13	3.13
Enterococcus faecalis	6.25	3.13
Escherichia coli NIHJ JC-2	〉25	12.5
Klebsiella pneumoniae DT-S	〉25	〉25
Shigella flexneri EW-10	〉25	6.25
Staphylococcus aureus FAD 209P	6.25	12.5
Staphylococcus aureus 1840	〉25	〉25
Streptococcus epidermis ATCC 1228	6.25	6.25

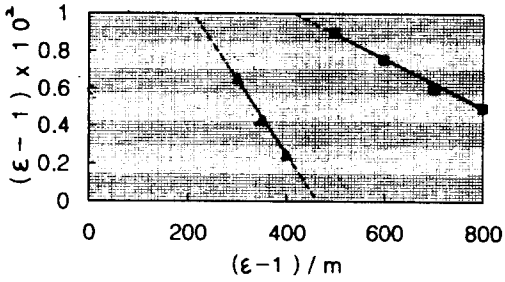

Fig. 2. Titration curve of mastoparan-B in DPPC (▲, Kd/n=5.0×10^{-4}) and DPPC-DPPG (■, Kd/n=1.2×10^{-4}) liposome.

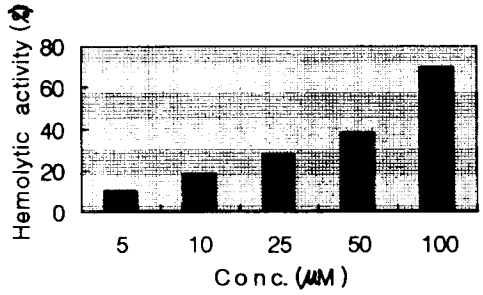

Fig. 3. The lysis of erythrocyte by mastoparan-B. An erythrocyte suspension was exposed to varying concentration of mastoparan-B.

dye release at an extremely low peptide concentration(1 μM) in neutral liposome (DPPC, Lee et al, 1986). In neutral liposome, mastoparan leaked dye with very high leakage ability. Mastoparan-B had high leakage ability but it did not reach 100% leakage even at high peptide concentration. In acidic liposome(DPPC-DPPG), the ability of the toxic peptide to release the dye was weaker than in neutral liposome, but the ability was in the same order(mastoparan>mastoparan-B) and did not reach 100% leakage all the case.

3.2 Binding affinity of toxic peptide

In an attempt to estimate the binding affinity of toxic peptide to lipid, the interactions of mastoparan and mastoparan-B with acidic and neutral liposome was examined. Comparison of the affinity parameter, Kd/n derived from the fluorescein intensity titration curve indicated that the affinity of mastoparan-B for DPPC-bilayer was smaller than that for DPPC-DPPG bilayer(Fig. 2).

3.3 Antibacterial activity

The result of antibacterial assay was presented in Table 1. Mastoparan had strong activity for gram-positive bacteria but weak one for gram-negative ones. Contrary to this, mastoparan-B had strong activity against gram-positive bacteria such as Staphylococcus aureus and Bacillus subtilus and a potent activity against gram-negative ones such as Escherichia coli and Shigella flexneri.

3.4 Interaction of toxic peptide with erythrocyte

Fig. 3 showed the hemolysis of human erythrocyte as a function of the con-

centration of toxic peptide. All the peptide lysed the erythrocyte very little up to 5 μM. Beyond 50 μM, mastoparan-B caused considerable lysis and about 70 % lysis was observed at 100 μM. These results suggested that the imino group of the indole moiety of Trp residue making up the hydrophobic region in amphiphilic helix was essential to the hemolytic activity of mastoparan-B.

The dye-release experiment showed that the membrane perturbation effect of mastoparan is larger than that of mastoparan-B in both neutral and acidic liposome. More hydrophobic mastoparan might lead to such difference. It should be noted that the ability of peptide-mediated dye release is much smaller in the acidic liposome than in neutral one. On the other hand, helix-forming ability and binding affinity for mastoparan-B were much larger in acidic liposome than in neutral one.

Our present study showed that the introduction of neutral hydrophilic amino acid into hydrophilic face changed the specificity for the antibacterial activity. These results will be useful for designing peptide that selectively activate various biological processes, for example, for understanding the specificity and generality of receptor-G-protein interaction. Furthermore, this study will be the base of understanding about the interaction of amphiphilic toxic peptide and cell membrane.

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Hornet 독액의 독성 Peptide와 Phospholipid 간의 생체환경적 상호작용

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Hornet venom의 독성 peptide인 mastoparan과 mastoparan-B를 solid phase peptide synthesis method로 합성한 후 이들과 phospholipid와의 상호작용, 이들의 항균 활성 및 용혈 활성을 조사하였다. 두가지 독성 peptide 모두 중성 liposome에서 저농도에서도 dye release를 유도할 수 있었다. 중성 liposome에 대한 mastoparan-B의 결합 친화도는 산성 liposome에서 보다 작았다. Mastoparan과 mastoparan-B는 두가지 모두 그람 양성 세균에 대하여 강한 항균 활성을 가지고 있었으나 그람 음성 세균에 대해서는 각각 약하거나 유력한 활성을 보였다. Mastoparan과 mastoparan-B는 5 μM의 낮은 농도에서도 적혈구를 분해시키는 독성을 보였다.