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References

1. Parker, A. J. *Chem. Rev.* **1969**, *69*, 1.
2. Tarkka, R. M.; Buncel, E. *J. Am. Chem. Soc.* **1995**, *117*, 1503.
3. (a) Abraham, M. H. *Pure & Appl. Chem.* **1985**, *57*, 1055. (b) Kondo, Y.; Sugitani, W.; Tokui, M.; Takagi, T. *J. Chem. Soc., Perkin Trans. 2* **1995**, 1049.
4. (a) Buncel, E.; Wilson, H. *Adv. Phys. Org. Chem.* **1977**, *14*, 133. (b) Buncel, E.; Um, I. H.; Hoz, S. *J. Am. Chem. Soc.* **1989**, *111*, 971. (c) Buncel, E.; Um, I. H. *J. Chem. Soc., Chem. Commun.* **1986**, 595.
5. (a) Neuvonen, H. *J. Chem. Soc., Perkin Trans 2* **1987**, 1723. (b) French, H. T. *J. Chem. Thermodynamics* **1987**, *19*, 1155. (c) Kovacs, H.; Laaksonen, A. *J. Am. Chem. Soc.* **1991**, *113*, 5596.
6. (a) Goiten, R.; Bruice, T. C. *J. Phys. Chem.* **1972**, *76*, 432. (b) Suh, J.; Kim, Y.; Lee, E.; Chang, S. H. *Bioorg. Chem.* **1986**, *14*, 33.
7. (a) Haak, Jan R.; Engberts, Jan B. F. N. *J. Org. Chem.* **1984**, *49*, 2387. (b) Henderson, J. W.; Haake, P. *J. Org. Chem.* **1977**, *42*, 3989.
8. March, J. *Advanced Organic Chemistry, 4th ed.*; Wiley & Sons: New York, 1992; p 358.
9. Jencks, W. P. *Catalysis in Chemistry and Enzymology*; McGraw-Hill: New York, 1972.
10. (a) Um, I. H.; Lee, S. J.; Kim, J. J.; Kwon, D. S. *Bull. Korean Chem. Soc.* **1994**, *15*, 473. (b) Lee, I.; Sohn, S. C.; Kang, C. H.; Oh, Y. J. *J. Chem. Soc., Perkin Trans. 2* **1986**, 1631.
11. Ménger, F. M.; Smith, J. H. *J. Chem. Soc.* **1972**, *94*, 3824.
12. (a) Castro, E. A.; Ibanez, F.; Santos, J. G.; Ureta, C. *J. Org. Chem.* **1993**, *58*, 4908. (b) Castro, E. A.; Ibanez, F.; Santos, J. G.; Ureta, C. *J. Chem. Soc., Perkin Trans. 2* **1991**, 1919.
13. (a) Um, I. H.; Kwon, H. J.; Kwon, D. S.; Park, J. Y. *J. Chem. Research (S), (M)* **1995**, in press. (b) Um, I. H.; Choi, K. E.; Kwon, D. S. *Bull. Korean Chem. Soc.* **1990**, *11*, 362.
14. (a) Um, I. H.; Jeon, J. S.; Kwon, D. S. *Bull. Korean Chem. Soc.* **1991**, *12*, 406. (b) Kwon, D. S.; Lee, G. J.; Um, I. H. *Bull. Korean Chem. Soc.* **1989**, *10*, 620.
15. Um, I. H.; Lee, G. J.; Yoon, H. W.; Kwon, D. S. *Tetrahedron Lett.* **1992**, *33*, 2033.
16. Miyaji, K.; Morinaga, K. *Bull. Chem. Soc. Jpn.* **1986**, *59*, 1695.
17. Eaton, G.; Pena-Nunez, A. S.; Symons, M. C. R. *J. Chem. Soc., Faraday Trans. 1* **1988**, *84*, 2181.
18. Treiner, C.; Tzias, P.; Chemla, M.; Poltoratskii, G. M. *J. Chem. Soc., Faraday Trans. 1* **1976**, *72*, 2007.
19. Taylor, D. F. G.; MacDonald, D. D. *Can. J. Chem.* **1976**, *54*, 2813.
20. (a) Westaway, K. *Can. J. Chem.* **1978**, *56*, 2691. (b) Weataway, K.; Lai, M. *Can. J. Chem.* **1989**, *67*, 345.
21. (a) Kolthoff, I. M.; Chantooni, M. K., Jr.; Bhowmilk, S. *J. Am. Chem. Soc.* **1968**, *90*, 23. (b) Coetzee, J. F.; Padmanabhan, G. R. *J. Am. Chem. Soc.* **1965**, *87*, 5005.

The Interaction of Mastoparan B from Venom of a Hornet *Vespa Basalis* with Phospholipid Matrices

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Mastoparan B (MP-B) that is a novel MP isolated from the hornet *Vespa basalis*, was studied as compared with MP, in terms of interaction with phospholipid bilayer and antimicrobial activity. MP-B has more hydrophilic amino acid residues in hydrophilic face of amphiphilic α-helical structure than MP. The both peptides exhibited considerably different effect on interaction with lipid bilayers, e.g. their conformation in the presence of acidic and neutral liposomes, dye-release ability from encapsulated liposomes, but on the whole the interaction mode was similar. On antimicrobial activity, MP had a strong activity against Gram-positive bacteria but no against Gram negative ones. Contrary to this, MP-B had a strong activity against Gram-positive and potent against Gram-negative ones. Since both peptides have almost same residues on the hydrophobic side, such more hydrophilic surface on the molecule seems to lead to the subtle change in its interaction with membranes, resulting in the alternation in its biological activity.

Introduction

Mastoparan (MP), a major component of the wasp venom, is a basic amphiphilic α-helical peptide composed of fourteen amino acid residues and a few of related peptides have been isolated from the natural source[1] (Figure 1). It possesses

	4 11 12
Mastoparan	Ile-Asn-Leu-Lys-Ala-Leu-Ala-Ala-Leu-Ala-Lys-Lys-Ile-Leu-NH_2
Mastoparan-M	Ile-Asn-Leu-Lys-Ala-Ile-Ala-Ala-Leu-Ala-Lys-Lys-Leu-Leu-NH_2
Mastoparan-T	Ile-Asn-Leu-Lys-Ala-Ile-Ala-Ala-Phe-Ala-Lys-Lys-Leu-Leu-NH_2
Mastoparan-A	Ile-Lys-Trp-Lys-Ala-Ile-Leu-Asp-Ala-Val-Lys-Lys-Val-Ile-NH_2
Mastoparan-II	Ile-Asn-Leu-Lys-Ala-Ile-Ala-Ala-Leu-Val-Lys-Lys-Val-Leu-NH_2
Mastoparan-X	Ile-Ans-Trp-Lys-Gly-Ile-Ala-Ala-Met-Ala-Lys-Lys-Leu-Leu-NH_2
Mastoparan-B	Ile-Lys-Leu-Lys-Ser-Ile-Val-Ser-Trp-Ala-Lys-Lys-Val-Leu-NH_2

Figure 1. Primary structure of mastoparans and mastoparan B.

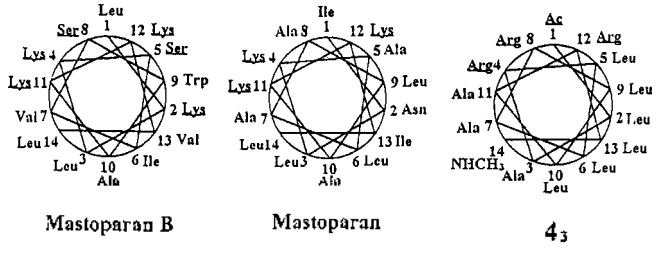

Figure 2. Helical wheel diagrams of MP, MP-B and a model peptide, 4_3.

a variety of biological activities such as activation of phospholipase A_2[2,3] and C,[3,4] erythrocyte lysis, mast cell degradation histamine release[1,2] and binding to calmodulin.[5] MP is also known to activate GTP-binding regulatory proteins (G-proteins) in a manner similar to that of G-protein-coupled receptors *in vitro*.[6] Structure-activity relationship studies, with the use of various natural and synthetic compounds, have shown that the amphiphilic α-helical structure is crucial to show such biological activity, with cationic amino acid residues on one side and with hydrophobic residues on the other side.[1,7] More recent study[8] has indicated that such structural feature is necessary but not itself sufficient to stimulate GTPase of G-protein; 1) the orientation of the positively charged amino acids relative to the N-terminus also seems to play and important role; 2) although hydrophobicity of hydrophobic moment of the peptides is related to their potency of ability to binding to G proteins, the relative contribution of the two parameters is unclear.

Recently, a novel MP, named mastoparan B (MP-B), has been isolated from the venom of the hornet *Vespa basalis* that is one of the most dangerous species of wasps found in Taiwan[9] (Figure 1). This peptide not only caused liberation of histamine from rat peritoneal mast cells, but also possesses an more potent hemolytic activity than MP. MP-B has been shown to elicit cardiovascular depressor[10] and to inhibit the growth of Gram-positive and -negative bacteria, at a minimum concentration of 19.5 μg/mL.[11] This peptide reveals the amphiphilic property as seen in the helical wheels (Figure 2). MP-B has more hydrophilic amino acid residues on the hydrophilic side of the amphiphilic structure (1-Leu, 5,8-Ser, 2,4,12-Lys, 9-Trp) as compared with those of MP (1-Ile, 2-Asn, 5,8-Ala, 4,12-Lys, 9-Leu), although both peptides have almost same residues on the hydrophobic side. Such more hydrophilic surface on the molecule might lead to the change in its interaction with membranes, resulting in the alternation in its biological activity. Studies on the interaction of MP-B with liposomes have not yet been the subject of any other reports. Thus in order to attain further information of the relationship between the hydrophilic side in the amphiphilic structure and the activity, we determined the conformation of MP-B by CD[31] measurement and its leakage and binding ability in the presence of neutral and acidic liposomes and its antimicrobial activity. The results were compared with MP[1] and an amphiphilic and antimicrobial α-helical peptides, 4_3[12,13] which are less and more in the hydrophobic regions of amphiphilic structure, respectively.

Materials and Methods

General. DPPC and DPPG were purchased from Sigma Chemical Co., St. Louis. Amino acids were purchased by the Peptide Institute, Osaka. Carboxyfluorescein from Eastman Kodak Co., Rochester was further purified by recrystallization from ethanol-water mixture. All other reagents used were of the highest grade available.

Purification and synthesis of MP-B. MP-B was synthesized according to Boc-chemical procedure starting from MBHA-resin by using a Biosearch model 9500 automatic peptide synthesizer with personal computer IBM PS/2 model 50Z. After HF-treatment, the crude peptide obtained was dissolved in 10% acetic acid and purified by Sephadex G-25 with 10% acetic acid and then HPLC chromatographies (a Shimadzu LC-6A system with ODS column, 20×250 mm) with a gradient system of water-acetonitrile containing 0.1% TFA. Amino acid analysis was performd on a Beckman System Gold amino acid analyzer after hydrolysis in 5.7 M HCl in a sealed tube at 110 ℃ for 24 hr. Analytical data obtained were as follows: Ala, 1.02 (1); Val, 2.23 (2); Leu, 3.00 (3); Ile, 0.96 (1); Ser, 1.97 (2); Lys, 3.78 (4). Molecular weight was determined by FAB-mass spectra using a JEOL SX-102 A: base peak, 1611. 060, calcd. for $C_{78}H_{138}O_{16}N_{20}$, 1611. 0600. MP was purchased from the Peptide Institute, Osaka and used without further purification. Peptide concentrations were determined from UV-absorbance of Trp in 8 M urea for MP-B and from quantitative amino acid analysis for MP.

Preparation of liposomes. Small unilamellar vesicles (SUVs) were prepared as follows. SUVs were prepared with a lipid composed of DPPC, DPPC-DPPG (3 : 1) as neutral and acidic vesicles, respectivly. Phospholipid (20 mg, about 25 mmol) was dissolved in chloroform (1 mL) and dried by breathing of nitrogen in a conical glass tube. The dried lipid was hydrated in 2 mL of 5 mM Tes buffer or Hepes buffer (pH 7.4) with repeated vortexed-mixing at 50 ℃ for 30 min using a Kaijo Denki ultrasonic disrupter model T-A-4280 and diluted to 25 mL with the same buffer (lipid concentration, about 1.0 mM). The small unilamellar vesicles obtained were used for the CD measurement.

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 0.1 M NaCl/5 mM Hepes buffer (pH 7.4) containing 100 mM carboxyfluorescein. After sonication, the mixture of uni-and multilamellar vesicles trapping carboxyfluorescein was subjected to gel-filtration through a Sephadex 4B column (1×20 cm) in 0.1 M NaCl/5 mM Hepes buffer (pH 7.4). Two milliliter fractions were collected and the solution of fraction number 7 that were just before the non-encapsulated dye-elution was collected to utilize for carboxyfluorescein re-

lease measurements. The lipid concentration of the fraction 7 was about 2.8 mM.

Circular dichroism spectra. Circular dichroism (CD) spectra were recorded on a JASCO J-600 spectropolarimeter using a quartz cell of 1 mm pathlength. Spectra in 5 mM Tes-buffer (pH 7.4) were measured at a peptides concentration of 100 mM. For measurements of the CD spectra of peptides in phospholipid liposomes, the peptides were dissolved directly in 5 mM Tes buffer (pH 7.4) containing 0.9 mM phospholipid liposomes. When the solution became cloudy after the addition of peptides to liposome solution, it was sonicated again to became clear with a both type sonicator at 25 ℃. To scan a scattering due to liposomes, the CD spectrum of liposomes was subtracted from that of the peptide in the presence of liposomes. All measurements were performed at 25 ℃ and the data were expressed in terms of the molar ellipticity. Spectra were analyzed as a linear combination of spectra for poly-L-lysine (M.W. 15,000-30,000) adopting the three well-known conformations depending on the solvent condition; α-helix, β-structure, and random coil.[14]

Leakage of carboxyfluorescein from liposomes. Leakage of liposome contents was determined with the fluorescence dye-release experiment from liposomes by the procedure of Weinstein *et al.*[16] with a minor modification.[15] Liposomes containing 100 mM 5(6)-carboxyfluorescein were prepared by sonication as described above. A liposome solution (50 μL) of fraction 7 obtained by passing through Sepharose 4B was added to a 2 ml of 5 mM Hepes buffer (pH 7.4) in the cuvette to give a final concentration of the peptide in phosphate buffer.

Fluorescence spectra. Fluorescence spectra were recorded on a JASCO FP-550A spectrofluorophotometer. The cuvette was placed in the heated cuvette holder of the fluorometer at 25 ℃ and the fluorescence intensity was continuously recorded. The intensity was monitored by fluorescence at 515 nm with exciting at 470 nm. The data were collected at 10 min after the addition of the peptides in the vesicle solution. For determination of the fluorescence intensity derived from 100% dye-release, 10 ml of Triton X-100 solution (20% in Hepes buffer) was added to dissolve the vesicles. The percentage of dye-release caused by the peptides was evaluated by the equation, $100\times(F-F_0)/(F_f-F_0)$, where F is the fluorescence intensity achieved by the peptides, F_0 and F_f are intensities of the fluorescence without the peptides and with Triton X-100 treatment, respectively.

Antimicrobial activity. The minimum inhibitory concentration (MIC) of the growth of microorganisms was determined by the standard agar dilution method using Muellar Hinton medium (Difco).

Spectroscopic titration of peptide with liposomes. Spectroscopic titration of peptides with liposomes was performed as reported by Surewicz and Epand.[17] Appropriate aliquots of DPPC or DPPC-DPPG (3 : 1) liposomes (1.0 mM) were successively added to a solution (2.0 mL) of peptides (6.24 μM) in Hepes buffer. After each addition of liposomes, the mixture was kept at 25 ℃ for 10 min to achieve the equilibration. From fluorescence spectra excited at 280 nm, the change of the maximum emission wavelength in fluorescence (λ/λ_0) of Trp was used to determine the affinity parameter Kd/n where Kd represents the dissociation constant of lipid peptide complex and n the number of binding site per lipid.

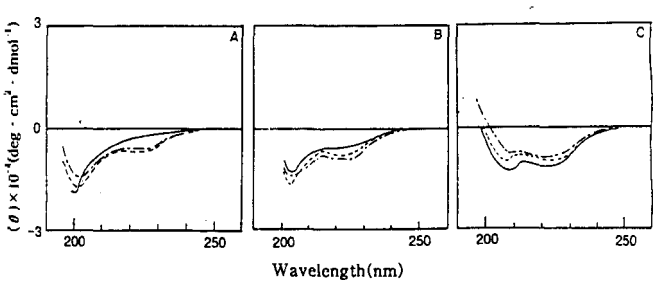

Figure 3. CD spectra of MP, MP-B and 4_3 in Tes buffer (A), in the presence of DPPC (B) and DPPC-DPPG (3 : 1) (C) liposomes. MP (---), MP-B (—) and 4_3 (-·--·-). Peptide and lipid concentrations are 0.1 mM and 1 mM, respectively.

Table 1. Secondary Structural Content of Peptides Determined by CD Spectra

Peptide	Media	α-helix	β-structure	Random
Mastoparan	Tes buffer	40 (%)	10 (%)	50 (%)
	DPPC	60	20	20
	DPPC-DPPG (3 : 1)	50	25	25
Mastoparan B	Tes buffer	15	10	75
	DPPC	35	10	50
	DPPC-DPPG (3 : 1)	70	15	15
4_3	Tes buffer	35	15	50
	DPPC	60	5	35
	DPPC-DPPG (3 : 1)	75	25	0

Results

CD analysis is a conventional method for studying conformational changes of polypeptides. The previous CD studies have shown that MP takes a random structure in buffer solution and α-helical structure in the presence of phospholipid bilayers,[7] although it also adopts α-helical structure at high ionic strength (more than 1 M NaCl) in aqueous solution.[18] In the present study, the spectra were measured in Tes-HCl buffer containing 100 mM NaCl and in the presence of DPPC and DPPC-DPPG (3 : 1) liposomes (Figure 3). In buffer solution, MP adopted a mainly random structure containing 40% α-helical structure, but MP-B still remained as a random structure (Table 1). In neutral and acidic liposomes, both MPs had double minimum peak around 205 and 222 nm region responsible for α-helical structure. Interestingly, MP-B took α-helical structure (35%) and the content of it was increased (70%) in acidic liposomes, but MP showed no such difference in both media (60% and 50%, respectively).

In order to study the interaction of MPs with model membranes, the ability of membrane perturbation was examined by measuring its effect of release of carboxyfluorescein from phospholipid liposomes in comparison with 4_3. Profiles of the dye-leakage from DPPC and DPPC-DPPG (3 : 1) liposomes caused by peptides are shown in Figure 4. Basic amphiphilic α-helical peptide 4_3 was employed as a reference. This pep-

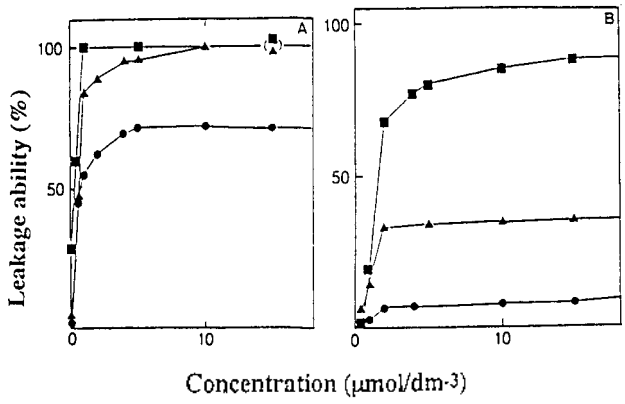

Figure 4. Leakage profiles of carboxyfluorescein from DPPC (A) and DPPC-DPPG (3 : 1) (B) liposomes by MP, MP-B and 4_3. MP (▲), MP-B (●) and 4_3 (■).

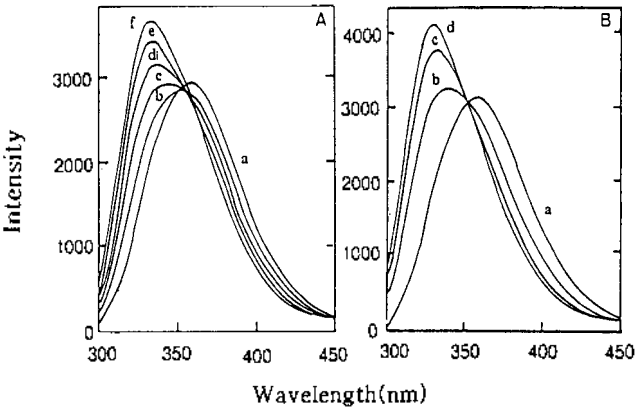

Figure 5. Fluorescence spectra of MP-B in the presence of DPPC (A) and DPPC-DPPG (3 : 1) (B) liposomes. Peptide concentrations; 6.24 μM. Lipid concentration: (a) 0, (b) 75, (c) 147, (d) 217, (e) 285, and (f) 352 μM.

tide has a strong antimicrobial activity against Gram-positive bacteria but a weak activity against Gram-negative bacteria and can induce a complete dye release at an extremely low peptide concentration (1 μM) in neutral liposomes.[12] In neutral liposomes, MP leaked dye with fairly high leakage ability (10 μM). MP-B has considerable leakage ability but it was saturated in the release of 80% and did not reach 100% leadage even at high peptide concentration. In acidic liposomes, the ability of the peptides to release the dye was in same order (4_3>MP>MP-B) but the ability were extremely low as compared with neutral liposomes, and did not reach complete leakages all the case.

In an attempt to attain the location of MPs into lipid bilayers and binding affinity to them, the interactions of MP-B with acidic and neutral liposomes were monitored by measuring Trp-fluorescence (Figure 5). Fluorescence spectra of MP-B in buffer solution showed an emission maximum at 360 nm. Upon the addition of both liposomes, the blue shifts to 325 nm were observed with increasing in lipid concentration, accompanying the increase in the maximum intensity. Such blue shifts indicate two possibilities: that the Trp residue in the peptide is located into lipid bilayer of a highly

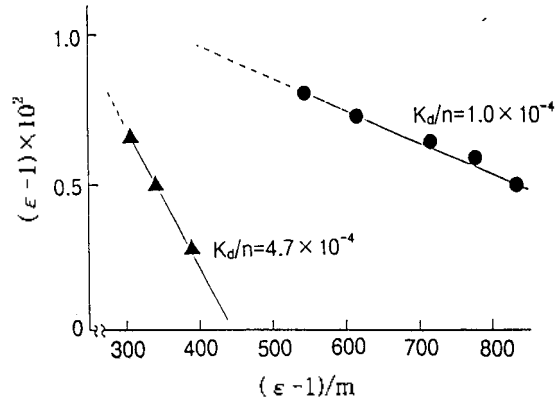

Figure 6. Titration curves of MP-B in the presence of DPPC (▲) and DPPC-DPPG (3 : 1) (●) liposomes. The ε represents λ_0/λ where the relative change of the maximum wavelength and the m represents lipid concentration as described in ref. 15.

Table 2. Antimicrobial activity of MP, MP-B, 4_3, Gramicidin S[a] and Apidaecin Ib[b]

Organism	Minimum inhibitory concentration ($\mu g ml^{-1}$)				
	MP	MP-B	4_3	GS	Apidaecin Ib
Enterococcus faecalis LS-101	6.25	3.13	3.13	3.13	>100
Staphylococcus aureus FAD 209P	6.25	12.5	3.13	3.13	>100
S. aureus 1840	>25	>25	3.13	3.13	>100
Streptococcus epidermidis ATCC 12228	6.25	6.25	3.13	3.13	>100
Bacilus subtilis PCI 219	3.13	3.13	3.13	3.13	>100
Escherichia coli NIHJ JC-2	>25	12.5	>25	>100	50
Shigella flexneri EW-10	>25	6.25	>25	>100	25
Klebsiella pneumniae DT-S	>25	>25	>25	50	100

[a]A cyclic decapeptides: cyclo(Val-Lys-Leu-D-Phe-Pro)$_2$. [b]Sequence: Gly-Asn-Asn-Arg-Pro-Val-Tyr-Ile-Pro-Gln-Pro-Arg-Pro-Pro-His-Pro-Arg-Leu-OH. [c]Method: Agar dilution method. Medium: Muellar Hinton agar (Difco). Inoculum size: 10^6 cells mL^{-1}

apolar amino acid cluster. The latter is, however, unlikely because the Trp residue in MP-X (see Figure 1) resembling MP-B as described later is located into lipid bilayer.[20] Comparison of the affinity parameters Kd/n derived from the fluorescence intensity titration curves indicates that the affinity of MP-B for DPPC bilayers is smaller than that for DPPC-DPPG bilayers (Figure 6).

The results of antimicrobial assay are listen in Table 2. MP and 4_3 had a strong activity against Gram-positive bacteria but no against Gram negative ones. Contrary to this, MP-B had a strong activity against Gram-positive bacteria such as *Staphylococcus aureus* and *Bacillus subtilis* and a potent against Gram-negative ones such as *Escherichia coli* and *Shigella sonnei*.

Discussion

The previous NMR study has indicated that mastoparan-

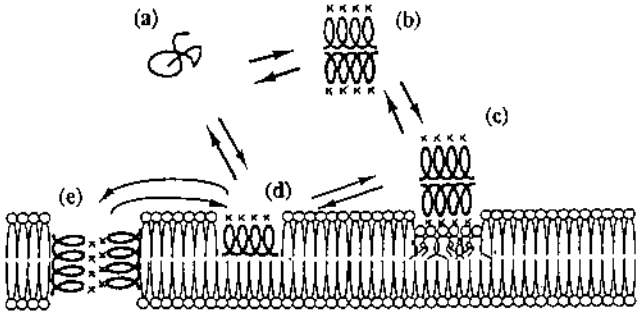

Figure 7. Proposed mechanism of lipid-peptide interaction by mastoparans. (a) a random form in bulk solution; (b) Aggregated form in bulk solution: (c) charge interaction of peptide with lipid bilayers; (d) partially penetrated form horizontally into lipid bilayers (no-release of dye encapsulated by liposomes); (e) a hole that possibly leaks dye.

X (MP-X, see Figure 1) takes an α-helical structure in the presence of perdeuterated DPPC vesicles in which the C-terminal 12 residues of 14 amino acid residues are present with the α-helical structure.[20] The interaction mode of peptide-lipid was considered that the hydrophilic side in the helix is present in neutral moiety of bilayer lipid head group and the hydrophobic side immersed into the membranes in the horizontal manner for the surface of lipid bilayer. The present study shows that MP and MP-B also take such amphiphilic structure in the presence of lipid bilayers.

Peptide-lipid interaction is often evaluated by the hydrophobicity and hydrophobic moment. The amphiphilicity of peptide is revealed by the hydrophobic moment.[21,22] The hydrophobicities and hydrophobic moments of MP, MP-B and MP-X are 0.05, 0.06, 0.01 and 0.22, 0.27, 0.21, respectively. Although the amphiphilicity of MP-B is relatively higher than MP-X, the difference among them is not so large. Therefore, MP-B is considered to interact with lipid bilayers in a manner similar to MP-X. Considerable difference between MP and MP-B, however, is observed for α-helical contents in the presence of acidic and neutral liposomes. The helical content of MP-B in acidic lipid bilayers is two times as much as that in neutral liposomes, while MP is slightly less in acidic liposomes. Since the hydrophobic region consists of almost same amino acid residues between both peptide, it seems that such difference come from that of hydrophilic region. The charge interaction between 2-Lys residues in MP-B and acidic lipid head group may play an important role in stabilizing the α-helical structure.

The present fluorescence study of MP-B also indicates that the peptide associates readily with the gel state of bilayers prepared from DPPC and DPPC-DPPG (3 : 1). This association is accompanied by the penetration of the indole ring of the tryptophan residue into a less polar environment, probably in the vicinity of the aliphatic chains. Since the Trp residue in MP-B is present on the hydrophobic part in the amphiphilic helices, it is reasonable to consider that MP-B interacts with lipid bilayers in such manner as shown by NMR-study as mentioned above.

The dye-release experiment shows that the membrane perturbation effect of MP is larger than that of MP-B in both neutral and acidic liposomes. More hydrophobic MP (the value of hydrophobicity for MP is larger than that for MP-B) might lead to such difference. It should be note that the ability of three peptides-mediated dye release is much smaller in the acidic liposomes than in neutral liposomes. On the other hand, helix-forming ability and binding affinity for MP-B are much larger in acidic liposomes than in neutral liposomes. Recent kinetic study of an amphiphilic α-helical peptide, magainin-induced release of carboxyfluorescein from vesicles of phosphatidylserine has shown that the cationic peptide interaction the negative surface of the membrane induces the transient bilayer destabilization through many steps.[23] From our results and those obtained by others, we can explain the different action mode of MPs between neutral and acidic membranes as follows (Figure 7). When the MP interacts with phospholipid membrane (Figure 7d), three of four cationic Lys residues are exposed to an aqueous phase, while the hydrophobic part of the molecule penetrates into the lipid bilayers; at that time, the peptide molecules are accumulated predominantly in the outer half of the bilayer (Figure 7e). In neutral liposomes, such outer leaflet accumulation of peptide molecules destabilize the alkyl chains in lipid bilayers and then the peptide can move to the inner leaflet. On the contrary, in the acidic liposomes, the charge interaction of cationic peptide-anionic lipid head group inhibits the transbilayer movements. The accumulation of peptides to the outer leaflet of bilayers (Figure 7d) may stabilize peptide-lipid interaction, which also inhibits to cause 100% dye-release as seen in Figure 4.

Amphiphilic structure has been found in biologically active peptide molecules.[24] In particular, the basic amphiphilic α-helical structure is considered to be one of the most important structural units for antimicrobial activity as found in naturally occurring peptides as melittin,[25] cecropin,[26] magainin[27] and dermaceptine[28] and in model peptides.[12,29,30] Both MP and MP-B exhibited strong antimicrobial activity, but considerable difference was observed on antimicrobial specificity; the former is active only against Gram-positive, while the latter is also active against Gram-negative bacteria as retaining the strong activity against Gram-positive one. We previously reported that in model peptides, Ac-(Leu-Arg-Ala-Leu)$_n$-NHCH$_3$ (n=2-4), 4_3 (n=3) is the highest antimicrobial activity among them, but only against Gram-positive bacteria.[12,29] Blondelle and Houghten[30] have also found that peptides of the 14- or 15-mer sequences have the high antimicrobial activity among the series of basic amphipathic peptides composed of 8 to 22 residues. Therefore it may be apparent that MPs should have a potent antimicrobial activity. However introduction of cationic amino acid residue, Lys, into hydrophobic face of amphiphilic peptides has shown an appreciable activity against Gram-negative bacteria in accompanying a drastical reduction in activity against Gram-positive bacteria, while less effect in activity was found upon introduction of Lys in hydrophilic face. Oppi *et al.*[8] have recently reported that from the experiment using various analogs replaced with cationic amino acid, the orientation of the positive charged amino acids relative to the N-terminus as well as that of the hydrophobic side chains appeared to play and important role in activation of certain G protein of MP. It has been also reported that MP-B possesses the more potent hemolytic activity than MP.[9] Our present study also shows that int-

roduction of neutral hydrophilic amino acid into hydrophilic face change the specificity for the antimicrobial activity. These results will be useful for designing peptides that selectively activate a variety of biological processes, *i.e.*, for understanding the specificity and generality of receptor-G-protein interaction. Such study using MP and MP-B is in progress.

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References

1. Nakajima, T.; Uzu, S.; Wakamatu, K.; Saito, K.; Miyazawa, T.; Yasuhara, T.; Tsukamoto, Y.; Fujino, M. *Biopolymers* **1986**, *25*, S115.
2. Hirai, Y.; Ueno, Y.; Yoshida, H.; Nakajima, T. *Biomed. Res.* **1983**, *1*, 185.
3. Argiolas, A.; Pisano, J. J. *J. Biol. Chem.* **1983**, *258*, 13697.
4. Okano, Y.; Takagi, H.; Tohmatsu, T.; Nakashima, S.; Kuroda, Y.; Saito, K.; Nozawa, Y. *FEBS Lett.* **1985**, *188*, 363.
5. Malencik, D. A.; Anderson, S. R. *Biochem. Biophys. Res. Commun.* **1983**, *114*, 50.
6. Higashijuma, T.; Uzu, S.; Nakajima, T.; Ross, E. M. *J. Biol. Commun.* **1988**, *263*, 6491.
7. Higashijima, T.; Burnier, J.; Ross, E. M. *J. Biol. Chem.* **1990**, *265*, 14176.
8. Oppi, C.; Wagner, T.; Crisari, A.; Camerini, B.; Valentini, G. P. T. *Proc. Natl. Acac. Sci. USA.* **1992**, *89*, 8268.
9. Ho, C-L.; Hwang, L. L. *Biochem. J.* **1991**, *274*, 453.
10. Ho, C-L.; Hwang, L-L.; Lin, Y-L.; Chen, C-T.; Yu, H-M.; Wang, K-T. *Eur. J. Pharmacol.* **1994**, *259*, 259.
11. Yu, H-M.; Wu, T-M.; Chen, S-T.; Ho, C-L.; Her, G-R.; Wang, K-T. *Biochem. Mol. Biol. Int.* **1993**, *29*, 241.
12. Lee, S.; Mihara, H.; Aoyagi, H.; Kato, T.; Izumiya, N.; Yamasaki, N. *Biochem. Biophys. Acta.* **1986**, *862*, 211.
13. Lee, S.; Mihara, H.; Aoyagi, H.; Kato, T.; Izumiya, N.; Ou, W. J.; Ito, A.; Omura, T.; Uzu, S.; Nakajima, T. *Peptide Chemistry* **1985** (ed., by Kiso, Y.), 317, *Protein Research Foundation* (Osaka).
14. Fukushima, K.; Muraoka, Y.; Inoue, T.; Shimozawa, R. *Biophys. Chem.*. **1988**, *30*, 237.
15. Suenaga, M.; Lee, S.; Park, N. G.; Aoyagi, H.; Kato, T.; Umeda, A.; Amako, K. *Biochem. Biophys. Acta.* **1988**, *981*, 143.
16. Weinstein, J. N.; Yoshikami, S.; Henkart, P.; Blementhal, R.; Hagins, W. A. *Science* **1977**, *195*, 489.
17. Surewicz, W. K.; Epand, R. M. *Biochemistry* **1984**, *23*, 6972.
18. Higashijima, T.; Wakamatsu, K.; Saito, K.; Fujino, M.; Nakajima, T.; Miyazawa, T. *Biochem. Biophys. Acta.* **1984**, *802*, 157.
19. Katsu, T.; Kuroko, M.; Morikawa, T.; Sanchika, K.; Fujita, Y.; Yamamura, H.; Uda, M. *Biochem. Biophys. Acta.* **1989**, *983*, 135.
20. Wakamatsu, K.; Okada, A.; Miyazawa, T.; Ohya, M.; Higashijima, T. *Biochemistry* **1992**, *31*, 5654.
21. Eisenberg, D.; Weiss, R. W.; Terwilligar, T. C. *Nature* **1982**, *299*, 371.
22. Eisenberg, D. *Annu. Rev. Biochem.* **1984**, *53*, 595.
23. Grant Jr. E.; Beeler, T. J.; Taylar, K. M. P.; Gable, K.; Roseman, M. A. *Biochemistry* **1992**, *31*, 9912.
24. Kaiser, E. T.; Kézdy, F. J. *Annu. Rev. Biophys. Biophys. Chem.* **1987**, *16*, 561.
25. Dowson, C. R.; Drake, A. F.; Helliwell, J.; Hider, R. C. *Biochim. Biophys. Acta.* **1978**, *510*, 75.
26. Steiner, H.; Andreu, D.; Merrifield, R. B. *Biochim. Biophys. Acta.* **1988**, *939*, 260.
27. Zasloff, M. *Proc. Natl. Acad. Sci. USA.* **1987**, *84*, 5449.
28. Mor, A.; Nguyen, V. H.; Delfour, A.; Migliore-Samour, D.; Nicolas, P. *Biochemistry* **1991**, *30*, 8824.
29. Mihara, H.; Kammera, T.; Yoshida, M.; Lee, S.; Aoyagi, H.; Kato, T.; Izumiya, N. *Bull. Chem. Soc. Jpn.* **1987**, *60*, 697.
30. Blondelle, S. E.; Houghten, R. A. *Biochemistry* **1992**, *31*, 12688.
31. The abbreviations recommended by the IUPAC-IUB commission of Biochemical Nomeuclature (*J. Biol. Chem.* **1972**, *247*, 977) have been used. Additional abbreviations: Boc, *t*-butyloxycarbonyl; TFA, trifluoroacetic acid; CD, circular dichroism; DPPC, dipalmitoyl-D,L-phosphatidylcholine; DPPG, dipalmitoyl-D,L-phosphatidylglycerol; Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; MP, mastoparan; Tes, N-tris(hydrodxymethyl)methyl-2-aminoethanesulfonic acid.