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Conformation and Biological Activity of Mastoparan B and Its Analogs I

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The mode of action of mastoparan B, an antimicrobial cationic tetradecapeptide amide isolated from the hornet *Vespa basalis*, toward phospholipid bilayers was studied with synthetic mastoparan B and its analogs with individual Ala instead of hydrophobic amino acids (1-Ile, 3-Leu, 6-Leu, 7-Val, 9-Trp, 13-Val, 14-Leu) in mastoparan B. Mastoparan B and its analogs were synthesized by the solid-phase method. Circular dichroism spectra showed that mastoparan B and its analogs adopted an unordered structure in buffer solution. In the presence of neutral and acidic liposomes, most of the peptides took an α-helical structure. The calcein leakage experiment indicated that mastoparan B interacted strongly with neutral and acidic lipid bilayers than its analogs. Mastoparan B also showed a more or less highly antimicrobial activity and hemolytic activity for human erythrocytes than its analogs. These results indicate that the hydrophobic face in the amphipathic α-helix of mastoparan B critically affect biological activity and helical contents.

Introduction

Mastoparan (MP), an antimicrobial cationic tetradecapeptide amide isolated from the venom of wasp (*Vespula lewisii*), is an amphiphilic α-helical peptide and its primary structure is Ile-Asn-Leu-Lys-Ala-Leu-Ala-Ala-Leu-Ala-Lys-Lys-Ile-Leu-NH_2.[1] MP toxin possesses a variety of biological activities such as activation of mast cell degradation histamine release,[2,3] phospholipase A_2[3,4] and C,[4,5] erythrocyte lysis and binding to calmodulin.[6] MP is also turned out to enhance the permeability of artificial membranes and biomembranes[7] and activate GTP-binding regulatory proteins (G-proteins) in a manner similar to that of G-protein-coupled receptors *in vitro*.[8] Structure-activity relationship studies with the use of various natural and synthetic compounds have shown that the amphiphilic α-helical structure with cationic amino acid residues on one side and hydrophobic residues on the other side is crucial to show such biological activity.[2,9] However, more recent study[10] has reported that such structural feature is necessary but not itself sufficient to stimulate GTPase of G-protein.

A similar peptide, namely mastoparan B (MP-B), was isolated from the venom of the hornet *Vespa basalis*[11] (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[12] and to inhibit the growth of Gram-positive and -negative bacteria, at a minimum concentration of 19.5 μg/mL.[13] Based on this relation, we have reported that MP-B has antimicrobial activity against both bacteria and leakage ability.[14] This peptide revealed the amphiphilic property as shown in the helical wheels.[14] NMR studies of MP-B in trifluoroethanol (TFE)-containing aqueous solution have indicated that residues 3-14 adopt an amphiphilic α-helical structure in which the residues with hydrophilic side chains (*i.e.* Lys-4, Ser-5, Ser-8, Lys-11, Lys-12) are located on one side and the residues with hydrophobic side chains (*i.e.* Leu-3, Ile-6, Trp-9, Ala-10, Val-13, Leu-14) located on the other side of the molecule.[15] The previous CD studies have shown that MP and MP-B take a random structure in buffer solution and α-helical structure in the presence of phospholipid bilayers,[9] but MP also adopts α-helical structure at high ionic strength (more than 1 M NaCl) in aqueous solution.[16] 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. The interaction of MP-B and its analogs with phospholipid bilayers have not reported as yet. Thus in order to attain further information of the relationship between the hy-

drophobic amino acids in MP-B and biological activity, we synthesized the seven analogs by replacing the hydrophobic amino acids in MP-B sequence with individual Ala. We also carried out their conformation by CD measurement, leakage in the presence of neutral and acidic liposomes and their hemolytic activity and antimicrobial activity.

Materials and Methods

General. Egg-yolk phosphatidylcholine (EYPC), egg-yolk phosphatidylglycerol (EYPG) and calcein were purchased from Sigma Chemical Co., St. Louis. Amino acids were purchased by the Watanabe Chemical Industries, LTD., Hiroshima. Phospholipid concentration was determined by an assay using the phospholipids-test reagent purchased from Wako Pure Chemical Industries (Osaka, Japan), and it was expressed in terms of phosphorus concentration. All other reagents used were of the highest grade available.

Synthesis and purification of MP-B and its analogs. MP-B and its analogs were synthesized according to Fmoc-chemical procedure starting from Fmoc-NHSAL resin using a Kokusan multi-peptide synthesis system. After TFA/thioanisole/ethanedithiol/*m*-cresol (20 : 5 : 3 : 1) treatment for 2 h to eliminate all the protecting groups and resin, the crude peptide obtained was dissolved in 10% acetic acid and purified by Sephadex G-25 with 10% acetic acid and then HPLC (a Waters 600 system with ODS column, 3.9× 300 mm) with a gradient system of water-acetonitrile containing 0.1% TFA. Amino acid analysis was carried out using a JASCO HPLC amino acid analysis system equipped with FP-210 spectrofluorometer as a detector after hydrolysis of the peptide in 5.7 M HCl in a sealed tube at 110 °C for 24 h. The amino acid analyses of the synthetic peptides were as follows: **1MP-B**; Ala 2.17 (2), Lys 3.98 (4), Leu 3.10 (3), Ser 1.88 (2), Val 1.99 (2). **3MP-B**; Ala 2.18 (2), Lys 3.97 (4), Leu 2.08 (2), Ser 1.87 (2), Val 1.91 (2), Ile 0.89 (1). **6MP-B**; Ala 2.19 (2), Lys 3.96 (4), Leu 2.14 (2), Ser 1.86 (2), Val 1.88 (2), Ile 0.73 (1). **7MP-B**; Ala 2.18 (2), Lys 3.94 (4), Leu 3.05 (3), Ser 1.84 (2), Val 0.99 (1), Ile 0.98 (1). **9MP-B**; Ala 2.26 (2), Lys 4.06 (4), Leu 3.10 (3), Ser 2.00 (2), Val 1.80 (2), Ile 0.79 (1). **13MP-B**; Ala 2.23 (2), Lys 4.03 (4), Leu 3.08 (3), Ser 1.88 (2), Val 0.94 (1), Ile 0.92 (1). **14MP-B**; Ala 2.06 (2), Lys 4.09 (4), Leu 2.11 (2), Ser 1.93 (2), Val 1.77 (2), Ile 0.85 (1). Numbers in parenthesis are theoretical values.

Molecular weight was determined by Fast atom bombardment mass spectra (FAB-MS) using a JEOL SX-102A. The FAB-MS data of the synthetic peptides were as follows: **1MP-B**: base peak, 1569.0, calcd. for $C_{75}H_{132}O_{16}N_{20}$, 1568.9. **3MP-B**: base peak, 1569.0, calcd. for $C_{75}H_{132}O_{16}N_{20}$, 1568.9. **6MP-B**: base peak, 1569.0, calcd. for $C_{75}H_{132}O_{16}N_{20}$, 1568.9. **7MP-B**: base peak, 1583.0, calcd. for $C_{76}H_{134}O_{16}N_{20}$, 1582.9. **9MP-B**: base peak, 1496.0, calcd. for $C_{70}H_{133}O_{16}N_{19}$, 1495.9. **13MP-B**: base peak, 1583.0, calcd. for $C_{76}H_{134}O_{16}N_{20}$, 1582.9. **14MP-B**: base peak, 1569.0, calcd. for $C_{75}H_{132}O_{16}N_{20}$, 1568.9.

Peptide concentrations for MP-B and its analogs were determined from UV-absorbance of Trp in 8 M urea .

Preparation of liposomes. Small unilamellar vesicles (SUVs) were prepared as follows. SUVs were prepared with a lipid composed of EYPC, EYPC-EYPG (3 : 1) as neutral and acidic vesicles, respectively. 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 (pH 7.4) with repeated vortexed-mixing at 50 °C 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 obtained SUVs were used for the CD measurement.

The unilamellar vesicles trapping calcein were prepared by the same method as described above except that the dried lipid (20 mg, 25 mmol) was hydrated in 2 mL of 0.15 M NaCl/20 mM Tes buffer (pH 7.4) containing 70 mM calcein. After sonication, the mixture of uni- and multilamellar vesicles trapping calcein was subjected to gel filtration through a Sephadex 4B column (1×20 cm) in 0.15 M NaCl/20 mM Tes 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 calcein release 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 the CD measurements 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 probe-type sonicator at 25 °C. 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 °C and the data were expressed in terms of the molar ellipticity. The α-helical contents were calculated from θ_{222} by Ben-Efraim *et al.*[17]

Leakage of calcein from liposomes. Leakage of liposome contents was determined using a minor modified the fluorescence dye-release experiment[18] rather than that proposed by Weinstein *et al.*[19] Liposomes containing 70 mM calcein 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 20 mM Tes 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 °C 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 vesicle solution. The fluorescence intensity of 100% dye-release was measured by adding 10 μL of Triton X-100 solution (20% in Tes buffer) in 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 con-

centration (MIC) of the growth of microorganisms was determined by the standard agar dilution method using Muellar Hinton medium (Difco).

Hemolytic assay. The buffy coat was removed by centrifugation of freshly collected human blood, and the obtained erythrocytes were washed three times with isotonic saline and stored at 4 °C. They were incubated with peptides at 37 °C in 10 mM Phosphate buffer containing 150 mM NaCl buffered saline, and then centrifuged. The absorbance in the supernatant was measured at 542 nm. The absorbance of the supernatant obtained by treatment of erythrocytes with 1% Triton X-100 was taken as 100%.

Results

CD study. To investigate the effect of hydrophobic amino acids of MP-B on biological activity and conformation, we designed MP-B analogs containing individual Ala residue instead of hydrophobic amino acids (1-Ile, 3-Leu, 6-Leu, 7-Val, 9-Trp, 13-Val, 14-Leu) in mastoparan B. The primary structures of peptides used in this study are shown in Figure 1. The compositions of amino acid in acid hydrolysates and the molecular weight of synthesized peptides coincidenced very well with the expected values as summarized in materials and methods sections.

The previous CD studies have shown that MP-B takes a random structure in buffer solution and α-helical structure in the presence of DPPC and DPPC-DPPG (3 : 1) phospholipid bilayers.[9] In order to investigate the conformations of MP-B and its analogs, in the present study, we measured CD spectra in Tes-HCl buffer containing 100 mM NaCl and in the presence of EYPC and EYPC-EYPG (3 : 1) liposomes as shown in Figures 2 and 3. In buffer solution, MP-B and its analogs adopted mainly random structure (Figures 2A and 3A). In the presence of neutral liposomes (Figures 2B and 3B), MP-B showed double minimum peak around 205 and 222 nm region corresponding to α-helix. The α-helical contents were increased in order of MP-B (43%)> 7, 13MP-Bs (30%)> 1MP-B(23%)> 14MP-B(10%)> 3, 6, and 9MP-Bs (trace). Interestingly, Ala replacement of amino acid residues locating to hydrophobic side in amphipathic helix decreased in the α-helical contents as compared to MP-B. In the presence of acidic liposomes (Figures 2C and 3C), the helical content progressively increased and its order was almost similar to that in neutral liposomes as follows: 7MP-B (65%)> 1, 13MP-Bs (59%)> MP-B (57%)≧ 6MP-B (55%), 14MP-Bs (54%)> 3MP-B (34%)> 9MP-B (27%). The hydrophobic interaction between the hydrophobic in-

MP-B	Leu-Lys-Leu-Lys-Ser-Ile-Val-Ser-Trp-Ala-Lys-Lys-Val-Leu-NH_2
1MP-B	Ala-Lys-Leu-Lys-Ser-Ile-Val-Ser-Trp-Ala-Lys-Lys-Val-Leu-NH_2
3MP-B	Leu-Lys-Ala-Lys-Ser-Ile-Val-Ser-Trp-Ala-Lys-Lys-Val-Leu-NH_2
6MP-B	Leu-Lys-Leu-Lys-Ser-Ala-Val-Ser-Trp-Ala-Lys-Lys-Val-Leu-NH_2
7MP-B	Leu-Lys-Leu-Lys-Ser-Ile-Ala-Ser-Trp-Ala-Lys-Lys-Val-Leu-NH_2
9MP-B	Leu-Lys-Leu-Lys-Ser-Ile-Val-Ser-Ala-Ala-Lys-Lys-Val-Leu-NH_2
13MP-B	Leu-Lys-Leu-Lys-Ser-Ile-Val-Ser-Trp-Ala-Lys-Lys-Ala-Leu-NH_2
14MP-B	Leu-Lys-Leu-Lys-Ser-Ile-Val-Ser-Trp-Ala-Lys-Lys-Val-Ala-NH_2

Figure 1. Primary structure of mastoparan B and its analogs.

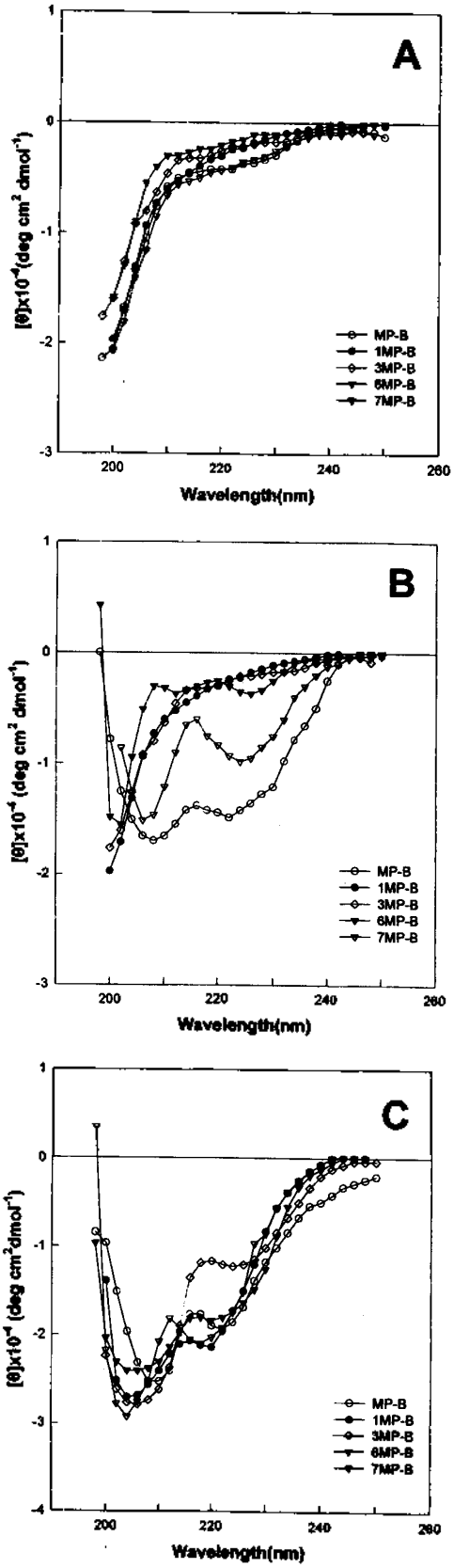

Figure 2. CD spectra of MP-B, 1MP-B, 3MP-B, 6MP-B, and 7MP-B in TES buffer (A) in the presence of EYPC liposomes (B) and EYPC-EYPG (3 : 1) liposomes (C). MP-B (○), 1MP-B (●), 3MP-B (◇), 6MP-B (▼), and 7MP-B (▽). Peptide and lipid concentrations are 0.1 and 1 mM, respectively.

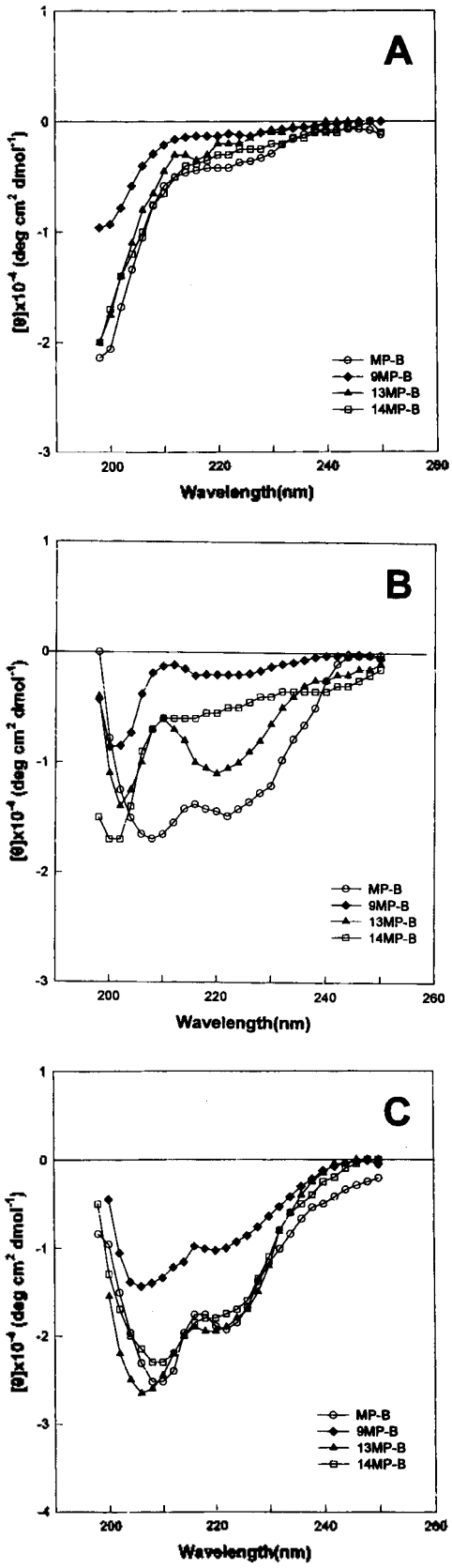

Figure 3. CD spectra of MP-B, 9MP-B, 13MP-B, and 14MP-B in TES buffer (A) in the presence of EYPC liposomes (B) and EYPC-EYPG (3 : 1) liposomes (C). MP-B (○), 9MP-B (◆), 13MP-B (▲), and 14MP-B (□). Peptide and lipid concentrations are 0.1 and 1 mM, respectively.

terior of phospholipid bilayers and hydrophobic amino acid residues is also regarded as an important factor in inducing the helical structure.

Leakage of liposome content. The ability of MP-B and its analogs to leak calcein from the inside of liposomes was evaluated to examine the perturbation of lipid bilayers induced by lipid-peptide interaction. Profiles of the dye release from EYPC and EYPC-EYPG (3 : 1) liposomes caused by peptides are shown in Figure 4. The addition of a 50 μM of peptides to calcein-containing EYPC liposomes induced the dye release in following order; MP-B> 3MP-B> 7, 13, 1 and 14MP-Bs> 6, 9MP-Bs (Figure 4A). Among the peptides, MP-B can leak the dye with the highest ability

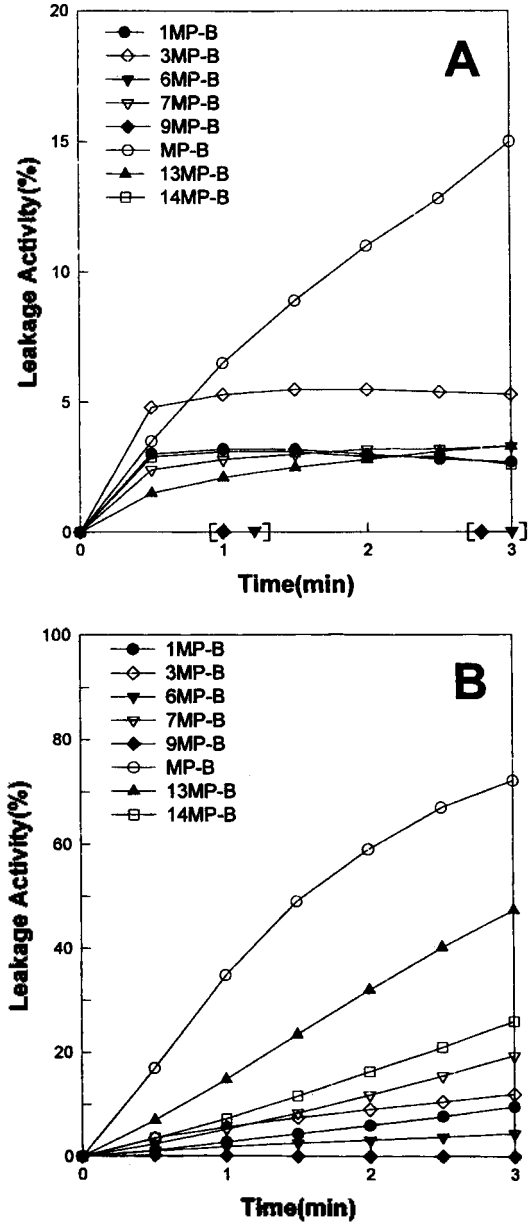

Figure 4. Time course of calcein release from EYPC (A) and EYPC-EYPG (3 : 1) (B) liposomes by MP-B and its analogs. Peptide concentrations are 50 μM (A) and 10 μM (B), respectively. MP-B (○), 1MP-B (●), 3MP-B (◇), 6MP-B (▼), 7MP-B (▽), 9MP-B (◆), 13MP-B (▲), and 14MP-B (□).

from the EYPC liposomes. MP-B reaches plateau with about 20% dye-release at about 50 μM and further release is not observed in the range of the concentration examined. Analogs of MP-B (50 μM) to release the dye were in same. Addition of a 10 μM of peptides to EYPC-EYPG (3 : 1) liposomes (Figure 4B), leakage ability is as follows; MP-B> 13MP-B> 14MP-B> 7MP-B> 3MP-B> 1MP-B> 6MP-B> 9MP-B. In acidic liposomes, the ability of MP-B and 13MP-B (10 μM) to release the dye increased as compared to other analogs but the leakage ability were somewhat better than neutral liposomes, and did not reach complete leakages all the case. These results suggest that the substitution of Ala to hydrophobic amino acid residues in amphiphilic structure is ineffective for membrane perturbation.

Antimicrobial activity. The minimum inhibitory concentration of the peptides is shown in Table 1. MP-B had a strong activity against Gram-positive and -negative bacteria. Contrary to this, the analogs of MP-B had a strong activity against Gram-positive bacteria such as *Bacillus subtilis* but gentle activity against Gram-negative ones. In general antimicrobial activity of the model peptides is less effective than that of MP-B. It is noted that the antimicrobial activity of peptides is closely parallel to their helical content as well as leakage ability.

Interaction of the peptides with red blood cells. Figure 5 shows the hemolysis of human erythrocytes as a function of the concentration of peptides. All the peptides lyse the erythrocyte very little up to 5 μM. Beyond 50 μM, MP-B and 7MP-B caused considerable lysis and about 70% and 30% lysis is observed at 100 μM, respectively. On the contrary, all the model peptides except for MP-B and 7MP-B scarcely lyse the erythrocytes at the concentration employed (50 μM). In particularly, 9MP-B resulted in a modest decrease in hemolytic activity relative to MP-B. These results appear to indicate that the imino group of the indole moiety of tryptophan residues making up the hydrophobic regions in amphiphilic helix are essential to the hemolytic activity of MP-B.

Discussion

NMR studies of MP-B in TFE-containing aqueous solution have indicated that residues 3-14 adopt an amphiphilic α-helical structure in which the residues with hydrophilic side chains (*i.e.* Lys-4, Ser-5, Ser-8, Lys-11, Lys-12) are located on one side and the residues with hydrophobic side chains (*i.e.* Leu-3, Ile-6, Trp-9, Ala-10, Val-13, Leu-14) located on the other side of the molecule.[15] The overall structural features were a quite similar to the conformation of mastoparan-X (MP-X) in perdeuterated DPPC vesicles even with the substitution made for eight residues with distinctly different hydrophobicity.[20] The previous CD study has indicated that MP and MP-B take an amphiphatic α-helical structure in the presence of artificial phospholipid bilayers, *i. e.* DPPC and DPPC-DPPG (3 : 1).[9] 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. MP-B and its analogs also take such amphiphilic structure in the presence of biological phospholipid 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] Using consensus hydrophobicity scales for amino acid residues offered by Eisenberg,[22] the hydrophobicities of MP-B, 1MP-B, 3MP-B, 6MP-B, 7MP-B, 9MP-B, 13MP-B and 14MP-B are −0.06, −0.08, −0.08, −0.10, −0.09, −0.07, −0.09 and −0.11, respectively. Additionally, the hydrophobic moments of MP-B, 1MP-B, 3MP-B, 6MP-B, 7MP-B, 9MP-B, 13MP-B and 14MP-B are 0.27, 0.29, 0.25, 0.24, 0.26, 0.27, 0.26 and 0.24, respectively. Because the difference of am-

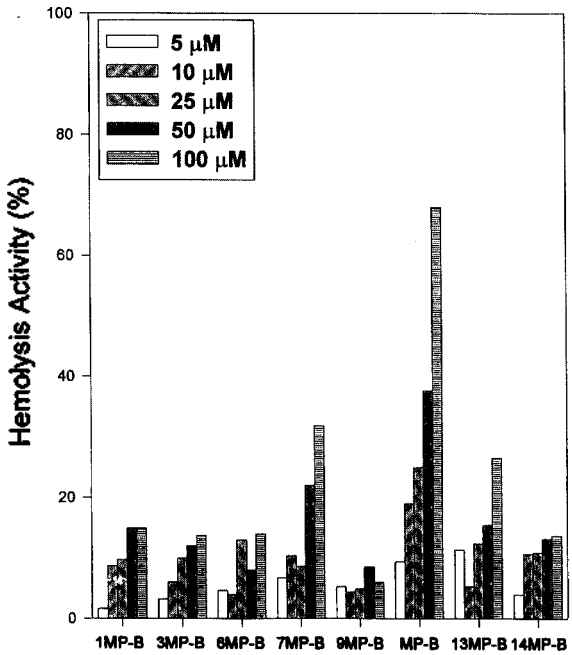

Figure 5. Erythrocyte lysis by MP-B and its analogs. An erythrocyte suspension was exposed to varying concentration of MP-B and its analogs. The amount of hemoglobin released was determined spectrophotometrically by measuring the absorbance of 540 nm. Complete lysis (100%) was obtained by the addition of Triton X-100 to the erythrocyte suspension.

Table 1. Antibacterial activity of MP-B and its analogsa

Organism	Mastoparan B	1MP-B	3MP-B	6MP-B	7MP-B	9MP-B	13MP-B	14MP-B
Staphylococcus aureus FDA 209P	50	>50	>50	>50	>50	>50	50	>50
S. epidermidie ATCC 12228	25	50	>50	>50	50	>50	25	50
Bacillus subtilis PCI 219	12.5	12.5	50	25	12.5	25	6.25	>50
Escherichia coli NIHJ JC-2	50	>50	>50	>50	>50	>50	50	50
Shigella flexneri EW-10	25	25	>50	50	25	25	12.5	>50

[a]Method: Agar dilution method, Medium: Muellar Hinton agar (Difco), Inoculum size: 10^6 cells mL^{-1}

phiphilicity among them is not so large, MP-B is presumed to be interacted with lipid bilayers in a manner similar to its analogs.

Considerable difference between MP-B and its analogs, however, is observed for α-helical contents in the presence of acidic (Figures 2C and 3C) and neutral liposomes (Figures 2B and 3B). The helical content of MP-B and its analogs in neutral lipid bilayers remarkably decreased as compared to acidic liposomes. Since the hydrophilic region consists of almost same amino acid residues between MP-B and its analogs, it seems that such difference comes from that of hydrophobic region. Therefore, the charge interaction between model peptides of MP-B and acidic lipid head group may not play an important role in stabilizing the α-helical structure.

The previous fluorescence study has indicated that MP-B associates readily with the gel state of bilayers prepared from DPPC and DPPC-DPPG (3 : 1).[9] 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 helixes, it is quite reasonable 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-B is larger than that for model peptides in both neutral and acidic liposomes. More hydrophobic MP-B (the value of hydrophobicity for MP-B is larger than that for model peptides) might lead to such difference. It should be noted that the ability of MP-B and its analogs-mediated dye release is much higher in acidic liposomes than in neutral liposomes. Similarly helix-forming ability for peptides is much larger in acidic liposomes than in neutral liposomes.

Amphiphilic structure has been found in biologically active peptide molecules.[23] 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,[24] cecropin,[25] magainin[26] and dermaceptine[27] and in model peptides.[28-30] As shown in Table 1, the MP-B and its analogs exhibited strong antimicrobial activity. As to both bacteria, the order of antimicrobial activity is approximately MP-B and 13MP-B> 1, 3, 6, 7, 9, and 14MP-Bs. The replacement of amino acid residues locating to hydrophobic side in amphipathic helix by Ala has shown a similar activity against Gram-positive bacteria in accompanying a little reduction in activity against Gram-positive bacteria. This is almost parallel with the leakage ability of encapsulated calcein from acidic liposomes, indicating that the antimicrobial activity of α-helical peptides against Gram-positive and -negative bacteria may be represented by the affinity of binding of the peptide to phospholipid bilayers. In a previous report, the 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.[28,29] Blondelle and Houghten[30] have also reported 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 turned out that MP-B and its analogs should have an antimicrobial activity. MP-B and model peptides have a little lytic activity against human erythrocyte. The naturally occurring α-helical peptides which serve as a defense system need to have broad-spectrum and strong activity against both bacteria. Therefore, MP-B is desired to cause no lysis of the mammalian cell. Ho and Hwang[11] have recently reported that MP-B show a marked hemolytic action on the red cells of several species of animals, including the guinea pig and the rat. Such considerable difference probably seems to be the cell specificity. Oppi *et al.*[10] 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 an important role in activation of certain G protein of MP. Our present study also shows that the helical content of the peptides is the significant index for their antimicrobial activity and hemolysis as well as leakage ability. The hydrophobic interaction between the hydrophobic interior of phospholipid bilayers and hydrophobic amino acid residues is also an important factor in inducing the helical structure. These results indicate that the appropriate hydrophobicity, including the proper orientation of hydrophilic (cationic) and hydrophobic groups in MP-B, is proposed for taking α-helical structure and exhibiting a variety of biological activity. In addition, these results will be useful for the structure-activity relationship of mastoparan toxins and designing peptides that selectively activate a variety of biological processes, *i.e.*, for understanding the specificity and generality of receptor-G-protein interaction. The studies on the receptor-G-protein interaction using MP-B and its analogs is in progress.

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Deposition of Ferroelectric $Pb(Zr_{0.52}Ti_{0.48})O_3$ Films on Platinized Silicon Using Nd:YAG Laser

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Lead zirconate titanate (PZT) thin films were deposited onto the $Pt/Ti/SiO_2/Si$ substrate by the pulsed laser deposition with the second harmonic wavelength (532 nm) of Nd:YAG laser. In order to determine the optimum conditions for the film deposition, the phase of the films were investigated as functions of ambient oxygen pressure, substrate temperature, and laser fluence. Also the chemical composition analysis was conducted for the PZT films deposited under various ambient oxygen pressure. When the distance between substrate and bulk PZT target is set to 20 mm, the optimum conditions have been determined to be 3 torr of oxygen pressure, 1.5 J/cm^2 of laser fluence, and 823-848($\pm$10) K range of substrate temperature. At these conditions, perovskite phase PZT films were obtained on platinized silicon. The chemical composition of the films is very similar to that of PZT bulk target. The physical structure of the deposited films analyzed by scanning electron microscopy shows a columnar morphology perpendicular to the substrate surface. Capacitance-Voltage hysteresis loop measurements show also a typical characteristics of ferroelectric thin film. The dielectric constant is found to be 528 for the 0.48 μm thickness of PZT thin film.

Introduction

Recently, thin films of ferroelectric materials with perovskite structure, such as $BaTiO_3$, $PbTiO_3$, and lead-zirconate-titanate (PZT) have attracted considerable attention due to their useful applications in various devices such as piezoelectric vibrator, surface acoustic wave devices, pyroelectric detectors, and nonvolatile random access memories.[1] Such applications require the deposition of these ceramic films onto silicon and the integration of the deposition techniques with semiconductor processing.[2]

A variety of processing modes including rf magnetron sputtering,[3] ion-beam sputtering,[4] electron cyclotron resonance (ECR) plasma stream,[5] sol-gel synthesis,[6] and pulsed laser deposition (PLD)[7] have been used to prepare ferroelectric thin films; among them, PLD has become a very

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