Biochimica et Biophysica Acta, 1027 (1990) 185–190
Elsevier

BBAMEM 74959

Interaction of wasp venom mastoparan with biomembranes

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(Received 14 March 1990)

Key words: Mastoparan; Melittin; Biologically active peptide; Membrane permeability; Membrane fluidity; Histamine release

Mastoparan-induced changes in the K^+ permeability of rat peritoneal mast cells, human erythrocytes, *Staphylococcus aureus* and *Escherichia coli* were examined. Mastoparan did not efficiently increase the K^+ permeability of cells except for *S. aureus*. The release of membrane phospholipids was also observed from *S. aureus* cells in the concentration range of the permeability enhancement. Mastoparan stimulated histamine release from mast cells, independently of a small efflux of K^+. Mastoparan became markedly effective to *E. coli* cells whose outer membrane structure was chemically disrupted beforehand, showing that the peptide can enhance the permeability of the cytoplasmic membranes of both Gram-positive and -negative bacteria. In experiments using liposomes, mastoparan increased the permeability of the liposomes composed of egg phosphatidylethanolamine and egg phosphatidylglycerol, which are the lipid constituents of the cytoplasmic membrane of *E. coli* cells, while it showed a weak activity to the liposomes composed of egg phosphatidylcholine and cholesterol. The latter result related closely to the fact that this peptide acted weakly on erythrocytes and mast cells in which acidic lipids constitute a minor portion. Mastoparan decreased the phase transition temperature of dipalmitoylphosphatidylglycerol liposomes, but it did not affect that of dipalmitoylphosphatidylcholine liposomes. These results indicate that mastoparan penetrated into membranes mainly containing acidic phospholipids and disrupted the membrane structure to increase the permeability. The action of the wasp venom mastoparan was compared with that of a bee venom melittin.

Introduction

Mastoparan is a toxic peptide found in the wasp venom, whose primary structure is represented as Ile-Asn-Leu-Lys-Ala-Leu-Ala-Ala-Leu-Ala-Lys-Lys-Ile-Leu-NH_2 [1,2]. Mastoparan stimulates the release of many biologically active substances from secretory cells [1]. It also enhances the permeability of planar lipid bilayer [1] or liposomal membranes [3]. Circular dichroism and nuclear magnetic resonance studies have shown that mastoparan has a strong affinity to phospholipid bilayer and holds an α-helical conformation in the bound state [4]. When the peptide forms the α-helical structure, positively charged groups (NH_2 groups of the N-terminus and three Lys residues) all lie on one side of the helix, while the other side is occupied by hydrophobic residues. This conformation in membrane is thought to play an important role in its toxic action [1].

Melittin, a bee venom, also interacts with membrane, forming a bent α-helical rod whose hydrophobic and hydrophilic amino acid residues are located in opposite sides of the structure [2]. This venom induces a wide variety of biochemical actions including hemolysis of red blood cells [2,5]. We have recently discussed the mechanism of hemolysis induced by melittin [6,7]. This peptide had an ability to stimulate the release of membrane fragments from erythrocytes, bringing about the intrusion of extra-cellular molecules of small size, leading to a colloid-osmotic hemolysis [6]. The peptide acted similarly on bacterial cytoplasmic membranes [7]. Because of the structural resemblance with melittin, it was supposed that mastoparan may also act on these biomembranes in a similar way. In the present study, using various types of cells (i.e., rat peritoneal mast cells, human erythrocytes, *Staphylococcus aureus* and *Escherichia coli*), we examined the action of mastoparan on biomembranes in detail. Although the peptide induced

Abbreviations: Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulphonic acid; Mops, 4-morpholinepropanesulphonic acid; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; DPPC, dipalmitoylphosphatidylcholine; DPPG, dipalmitoylphosphatidylglycerol.

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the efflux of K^+ from *S. aureus* cells resulting from the release of membrane phospholipids, it did not enhance the K^+ permeability of mast cells, erythrocytes and *E. coli* cells significantly. The selective activity of mastoparan to *S. aureus* cells was explained in view of an affinity of this peptide to the negatively charged membrane phospholipids.

Materials and Methods

Materials. The sources of materials were as follows: mastoparan from Peptide Institute, Inc., Japan; melittin (product number M2272; lot number 95F-4024), cholesterol, DPPC and DPPG from Sigma, U.S.A.; egg PC from The Green Cross Corporation, Japan; egg PE and egg PG from Lipid Products, Britain. All other chemicals were of analytical reagent grade.

Erythrocytes and bacteria. Human erythrocytes were washed twice with buffer (0.15 M NaCl/5 mM Hepes-NaOH, pH 7.4) and suspended in this buffer at the final concentration of 1% hematocrit. The number of cells was $1 \cdot 10^8$ cells/ml. The total amount of phospholipids contained in this cell suspension was 60 μM in egg PC equivalent. *S. aureus* FDA 209P and *E. coli* K12 strain W3110 were grown as reported previously [7]. Washed *S. aureus* cells were suspended in 100 mM choline chloride/50 mM Mops-Tris (pH 7.2) at $5 \cdot 10^9$ cells/ml (0.6 mg cell protein/ml), whose phospholipid concentration was the same as that of erythrocytes. *E. coli* cells were suspended in the same buffer used in *S. aureus* at $5 \cdot 10^9$ cells/ml (0.7 mg cell protein/ml). Protein content was determined by the method of Lowry et al. [8]. Cells were incubated with a peptide at 37°C for 30 min. The amount of K^+ efflux was measured with a K^+ ion-selective electrode [9]. The total amount of K^+ was determined by disrupting cells with a surfactant Triton X-100 or cetyltrimethylammonium bromide [10]. Hemolysis was estimated by measuring the absorbance at 540 nm [6]. The viability of cells was determined by counting colonies [7]. The amounts of phospholipids and lipopolysaccharides released from cells were analysed after cells had been centrifuged at $14000 \times g$ for 1 min (erythrocytes) or at $14000 \times g$ for 3 min (bacteria) [7]. To disrupt the outer membrane structure of *E. coli*, cells were treated with 150 mM Tris-HCl (pH 7.2)/1 mM EDTA at 37°C for 2 min [7]. Because EDTA was toxic to cells [11], about 30% of cells was killed by this procedure.

Mast cells. Mast cells were collected from the peritoneal cavities of male Wistar rats (300–350 g) and purified using Percoll as reported previously [12]. The mast cells were suspended in a K^+-free buffer solution comprising 0.154 M NaCl, 0.9 mM $CaCl_2$, 5.6 mM glucose, 0.01% bovine serum albumin and 5 mM Hepes-NaOH (pH 7.4) at $3 \cdot 10^5$ cells/ml. Then, a peptide was added and was incubated at 20°C for 5 min. Because a great deal of spontaneous efflux of K^+ was observed at 37°C, this experiment was carried out at the lower temperature of 20°C. After cells were centrifuged out, the amounts of K^+ efflux and histamine release were determined. The histamine was determined fluorometrically after conversion of histamine to the fluorescent product by reaction with *o*-phthalaldehyde as reported previously [13,14].

Liposomes. Lipids of erythrocytes and *E. coli* cells were extracted according to the methods in the literature [15,16]. Liposomes were prepared by the method of reverse-phase evaporation [9]. The inner aqueous phase of liposomes contained 0.15 M KCl/5 mM Hepes-NaOH (pH 7.4). Washed liposomes were suspended in 0.15 M NaCl/5 mM Hepes-NaOH (pH 7.4).

Fluorescence polarization. Peptide-induced changes in the phase transition temperature of DPPC or DPPG liposomes were measured by the fluorescence polarization technique [9]. Diphenylhexatriene (1 mol% of lipid) was used as a fluorescence probe. DPPC or DPPG containing diphenylhexatriene was swollen in a buffer solution of 0.15 M NaCl/5 mM Hepes-NaOH (pH 7.4) at 55°C to prepare multilamellar liposomes. A small aliquot of the liposomes was pipetted and suspended in the same buffer at the final concentration of 100 μM of lipid. Then a peptide (20 μM) was added to this suspension, and the suspension was briefly sonicated (bath-type, Bransonic B-220, 125 W) for about 10 s at 55°C to obtain a homogeneous dispersion of the peptide. Fluorescence polarization was measured by excitation at 360 nm and emission at 430 nm. The degree of polarization (P) was calculated as reported previously [9].

Results

Action of mastoparan on biological membranes

Fig. 1 shows mastoparan-induced K^+ efflux, hemolysis and the release of lipid components from human erythrocytes, *S. aureus* and *E. coli*. Although mastoparan stimulated remarkably the efflux of K^+ from *S. aureus* cells, it acted weakly on both erythrocytes and *E. coli* cells. Also, hemolysis took place little from erythrocytes in accordance with a small efflux of K^+. So far, a permeability enhancement of membrane induced by amphipathic peptides has been discussed from the viewpoint of the formation of a disordered region in the lipid bilayer, or of their channel-forming abilities [1,2]. However, we have recently found that amphipathic peptide melittin stimulates the release of membrane phospholipids out of cells, resulting in a permeability enhancement concurrently [7]. Thus, we were interested in whether mastoparan also increased a permeability by the release of phospholipids. It was observed that mastoparan stimulated the release of phospholipids from *S. aureus* cells in the same concentration range of the K^+ efflux, but it did not effi-

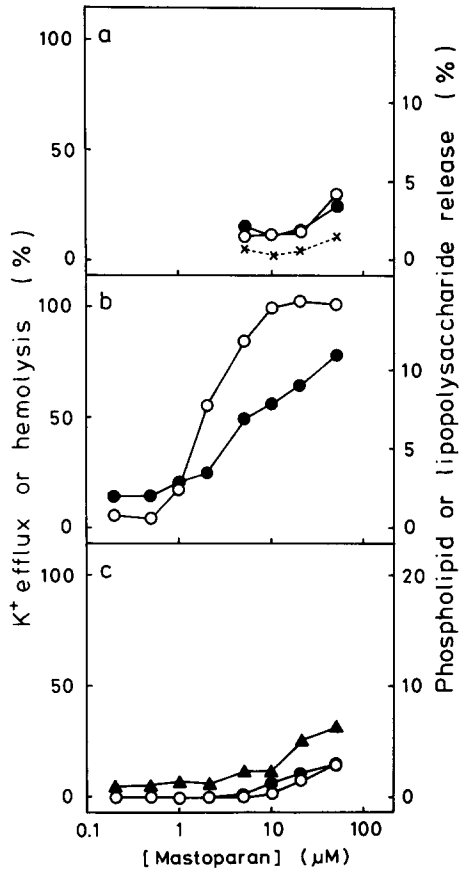

Fig. 1. The dose-response curves of K^+ efflux (○), hemolysis (×) and phospholipid (●) or lipopolysaccharide (▲) release from (a) erythrocytes, (b) *S. aureus* and (c) *E. coli* cells induced by mastoparan. Erythrocytes were suspended in 0.15 M NaCl/5 mM Hepes-NaOH (pH 7.4) at $1 \cdot 10^8$ cells/ml. *S. aureus* and *E. coli* cells were suspended in 100 mM choline chloride/50 mM Mops-Tris (pH 7.2) at $5 \cdot 10^9$ cells/ml. Cells were incubated with mastoparan at 37°C for 30 min. Phospholipids and lipopolysaccharides released from cells were analysed after cells had been removed at 14000×*g* for 1 min (erythrocytes) or at 14000×*g* for 3 min (bacteria).

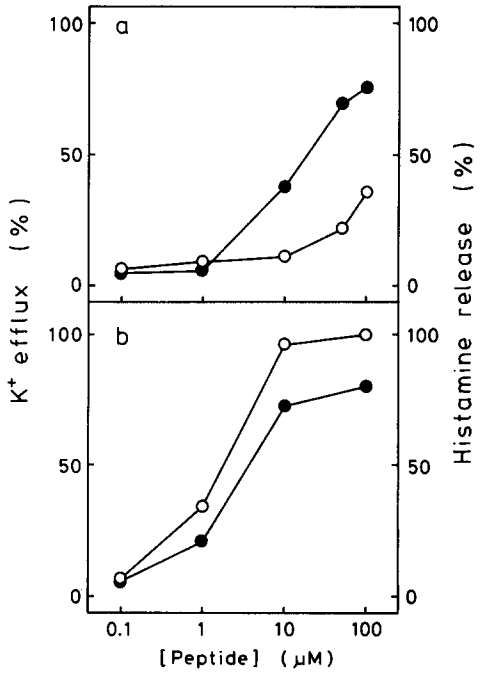

Fig. 2. The efflux of K^+ (○) and the release of histamine (●) from mast cells induced by (a) mastoparan and (b) melittin. Rat peritoneal mast cells were suspended in a K^+-free buffer solution comprising 0.154 M NaCl, 0.9 mM $CaCl_2$, 5.6 mM glucose, 0.01% bovine serum albumin and 5 mM Hepes-NaOH (pH 7.4) at $3 \cdot 10^5$ cells/ml. The amounts of K^+ efflux and histamine release were determined after cells were incubated with peptide at 20°C for 5 min.

ciently increase from both *E. coli* cells and erythrocytes (Fig. 1). These results showed evidently that the mode of action of mastoparan on *S. aureus* cells coincides well with that of melittin. However, the action spectra of the two peptides on the cells differed largely. Although mastoparan acted only on *S. aureus* cells as described above, melittin exerted strong effects on both erythrocytes and *S. aureus* cells [7]. Now, we compared the action of mastoparan and melittin on mast cells. As shown in Fig. 2, mastoparan did not greatly increase the efflux of K^+ from mast cells, while melittin enhanced markedly, in accordance with the results of erythrocytes. Histamine release was clearly observed with both peptides, indicating that histamine released irrespective of the occurrence of K^+ efflux.

We further investigated changes in the viability of cells induced by mastoparan. The peptide decreased remarkably the viability of *S. aureus* cells, but scarcely acted on *E. coli* cells (Fig. 3), in proportion to the K^+ efflux. Lower susceptibility to *E. coli* cells may be attributed to the different membrane structure. In the case of Gram-negative bacteria such as *E. coli*, an extra outer membrane exists outside the cytoplasmic membrane and forms a permeability barrier against various drugs [11,17]. The fact that mastoparan did not release lipopolysaccharide remarkably (Fig. 1c) shows its weak ability to disrupt the outer membrane structure. After being treated with Tris-EDTA to disrupt the outer membrane structure intensively [11,17], *E. coli* cells

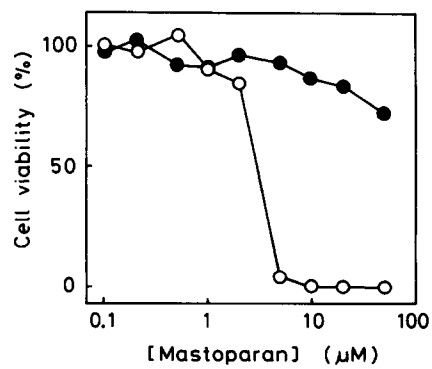

Fig. 3. Changes in the viability of *S. aureus* (○) and *E. coli* (●) cells after treatment with mastoparan. Cell viability was measured under the same conditions of the K^+ permeability measurements.

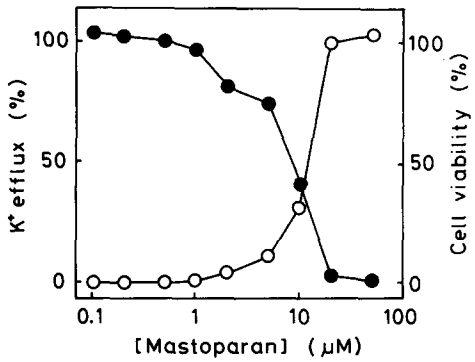

Fig. 4. Action of mastoparan on Tris-EDTA-treated *E. coli* cells. The dose-response relations of cell viability (●) and K^+ efflux (○) are shown. Cells were incubated with mastoparan at 37°C for 30 min.

became markedly sensitive to mastoparan, (Fig. 4), indicating that the peptide can enhance the permeability of the cytoplasmic membranes of both Gram-positive and -negative bacteria.

Action of mastoparan on liposomal membranes

In order to examine whether the action of mastoparan was dependent on the lipid constituents of membrane, we extracted lipids from erythrocytes and *E. coli* cells to prepare liposomes by the method of reverse-phase evaporation [9]. The lipid extracted from *E. coli* cells did not contain lipopolysaccharide, but originated mainly from the cytoplasmic membrane. As shown in Table I, mastoparan enhanced greatly the efflux of K^+ from the liposomes prepared with *E. coli* lipids, but it acted weakly on the liposomes prepared from erythrocytes. These results correlated well on the permeability changes in intact cells. Here, we are interested in whether all of the action of mastoparan on biomembranes can be reproduced by using the liposomes of egg yolk phospholipids. Although the peptide increased greatly the permeability of the liposomes prepared from egg PC alone, it did not act efficiently on the liposomes composed of egg PC/cholesterol. The presence of cholesterol reduced the action of mastoparan, which corresponded well with the low sensitivity of this peptide to erythrocytes and mast cells. Mastoparan increased markedly the permeability of egg PE/egg PG liposomes, in a similar way to the cytoplasmic membrane of *E. coli*. For comparison, the action of melittin was also investigated. Melittin is known to increase the permeability of both erythrocyte and bacterial cytoplasmic membranes [7]. In accordance with this fact, melittin increased the permeability of all types of liposomes prepared above. Table I summarizes the percentage of K^+ efflux induced by the peptides.

TABLE I

Percentage of peptide-induced K^+ efflux from liposomes

Liposomes were suspended at the following lipid concentrations: erythrocyte lipid (300 μg/ml); *E. coli* lipid (300 μg/ml); egg PC (300 μM); egg PC/cholesterol (300/300 μM); egg PE/egg PG (250/50 μM). The concentration of peptide added was 20 μM. Percentage effused within 5 min was calculated. Measurements were made at 28°C.

	% peptide-induced K^+ efflux	
	Mastoparan	Melittin
Erythrocyte lipid	40	100
E. coli lipid	95	100
Egg PC	100	100
Egg PC/cholesterol	10	85
Egg PE/egg PG	100	100

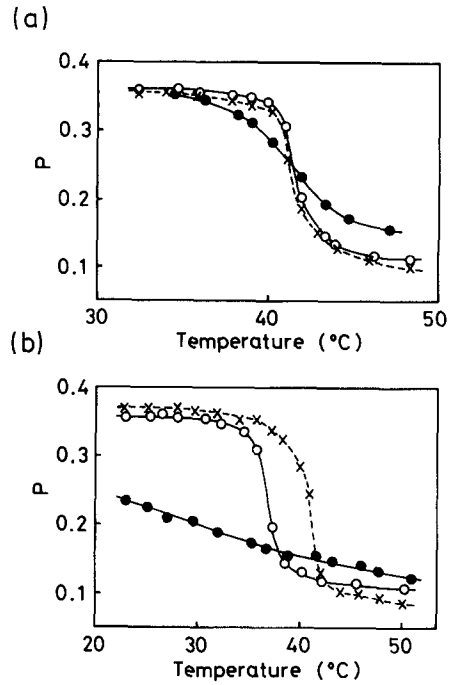

Fig. 5. Changes in the degree of polarization (P) of (a) DPPC or (b) DPPG liposomes before and after addition of mastoparan or melittin. Liposomes (100 μM) alone (×) and the liposomes containing 20 μM mastoparan (○) or melittin (●) were suspended in 0.15 M NaCl/5 mM Hepes-NaOH (pH 7.4).

Finally, we examined the effects of peptides on the phase transition temperature of liposomes (Fig. 5). Mastoparan decreased the phase transition temperature of DPPG liposomes, but it did not affect that of DPPC liposomes at all. Melittin broadened the phase transition profiles of both DPPG and DPPC liposomes.

Discussion

The present study shows that mastoparan enhanced the K^+ permeability of *S. aureus* cells, but it did not increase remarkably that of mast cells, erythrocytes or *E. coli* cells. The release of membrane phospholipids was also observed from *S. aureus* cells under the conditions of the efflux of K^+, indicating that mastoparan

enhanced the permeability of *S. aureus* cells by disrupting membrane structure as in the case of melittin [7]. We pretreated *E. coli* cells with Tris-EDTA to disrupt the outer membrane structure existing in Gram-negative bacteria. Thus treated *E. coli* cells became strongly sensitive to the peptide, showing that mastoparan can act on the cytoplasmic membranes of both Gram-positive and -negative bacteria.

The previous study [1] has shown that mastoparan increased the membrane conductance of a planar lipid bilayer, inducing the penetration of Ca^{2+}, Na^+ or K^+ ions non-specifically. It was supposed that such a permeability enhancement of membrane triggered histamine release [1]. In mast cells, however, the efflux of K^+ did not efficiently occur, irrespective of a marked release of histamine. It is worth noting here that compound 48/80, a famous histamine-releasing agent, also increased the conductivity of planar membrane [18], but it did not sufficiently increase the K^+ efflux from mast cells [19] as in the case of mastoparan. Thus, there is a tendency that the planar membrane is more easily damaged than the biomembrane. A small efflux of K^+ from mast cells implies that a histamine secretion proceeds without disrupting the membrane structure extensively. Therefore, mastoparan is expected to secrete histamine from rat peritoneal mast cells by a non-cytotoxic process similarly to the case of compound 48/80 [20], while the action of melittin on mast cells is cytotoxic. Recent studies [21–24] have suggested that mastoparan and compound 48/80 activate a GTP-binding protein (G protein), increasing phospholipase C activity to induce histamine secretion.

We examined the effects of peptides on the phase transition temperature of phospholipid to evaluate their abilities to enter into the lipid bilayer. This experiment is based on the following view: When an amphipathic peptide penetrated into the lipid bilayer, an expansion of lipid packing occurs in the vicinity of the polar heads of lipids to stimulate the movement of the interior acyl chains, leading to a decrease in the phase transition temperature [10,25]. In the case of mastoparan, a marked decrease in the phase transition temperature of DPPG liposomes was observed, while that of DPPC liposomes was not affected at all. These results correlated well with the fact that mastoparan penetrated into bacterial cytoplasmic membranes being rich in negatively charged phospholipids, while it did not act on erythrocytes or mast cells whose membrane components consist of zwitterionic phospholipids predominantly. However, we have also observed that mastoparan increased the permeability of the liposomes composed of a zwitterionic lipid, egg PC (Table I), conflicting with the above explanation. Here, it should be pointed out that a decrease in the phase transition temperature reflects the ability of peptide to penetrate into the gel phase of membrane. Although mastoparan was not able to enter into the gel phase of DPPC membrane, it could penetrate into the liquid phase of egg PC membrane to increase the permeability. We further consider that mastoparan can not effectively intrude into cholesterol-containing membrane, because the addition of cholesterol suppresses considerably the fluidity of the liquid phase of PC membrane [26,27]. Thus, the peptide could not efficiently increase the permeability of liposomes composed of egg PC/cholesterol, erythrocytes and mast cells. Recent studies [10,28] have also supported the above consideration.

As for melittin, the broadening of the phase transition temperature of both DPPG and DPPC liposomes was observed in accordance with the previous results [29,30], indicating that melittin penetrated into liposomes perturbs the membrane structure strongly [31]. Owing to such a strong membrane perturbation without any selectivity for phospholipids, melittin increased the permeability of all kinds of liposomes, mast cells, erythrocyte and bacterial cytoplasmic membranes.

Acknowledgements

We are grateful to Ms. Keiko Komagoe for her careful drawing the figures. This work was supported in part by a Grant-in-Aid for Scientific Research (No. 01571178) from the Ministry of Education, Science and Culture of Japan.

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