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*Published in:*  
Biochemistry

*DOI:*  
[10.1021/bi000917a](https://doi.org/10.1021/bi000917a)

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*Document Version*  
Publisher's PDF, also known as Version of record

*Publication date:*  
2000

[Link to publication in University of Groningen/UMCG research database](#)

*Citation for published version (APA):*

Moll, G. N., Brul, S., Konings, W. N., & Driessen, A. J. M. (2000). Comparison of the membrane interaction and permeabilization by the designed peptide Ac-MB21-NH2 and truncated dermaseptin S3. *Biochemistry*, 39(39), 11907 - 11912. <https://doi.org/10.1021/bi000917a>

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# Comparison of the Membrane Interaction and Permeabilization by the Designed Peptide Ac-MB21-NH<sub>2</sub> and Truncated Dermaseptin S3<sup>†</sup>

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Received April 24, 2000; Revised Manuscript Received June 30, 2000

**ABSTRACT:** Ac-MB21-NH<sub>2</sub> (Ac-FASLLGKALKALAKQ-NH<sub>2</sub>) and dermaseptin S3(1–16)-NH<sub>2</sub> (ALWKNMLKGIGKLAGK-NH<sub>2</sub>) are cationic amphipathic peptides with antimicrobial activity against a broad spectrum of microorganisms including various fungi. The interaction of the peptides with liposomes was studied by exploiting the tryptophan fluorescence of F1W-Ac-MB21-NH<sub>2</sub> and dermaseptin S3(1–16)-NH<sub>2</sub>. Spectral analysis and the use of quenchers indicate that the tryptophans of both peptides insert more deeply in anionic than in zwitterionic liposomes. Membrane insertion correlates with the formation of an  $\alpha$ -helical peptide structure. Both peptides permeabilize liposomes composed of anionic, cylindrical phospholipids more efficiently than liposomes formed of zwitterionic, conic (phospho)lipids.

Eukaryotic and prokaryotic organisms produce antimicrobial peptides to defend themselves against invading pathogenic organisms or to eliminate organisms that compete for the same niche. Many of these antimicrobial peptides are composed of 10–46 amino acids and permeabilize the plasma membrane of the target organism. Some of these peptides have a very narrow spectrum of activity, while others exert their activity on a broad range of organisms. Especially the latter might be interesting as food additives or as new antibiotics. The basis of the specificity is not understood. Many microbicidal peptides, among which dermaseptins (1, 2), have been isolated from frog skin (3). Dermaseptins kill various microorganisms including Gram-positive and -negative bacteria, protozoa among which *Leishmania mexicana* and *Plasmodium falciparum*, and yeasts and filamentous fungi, among which the pathogenic *Aspergillus fumigatus* and *Aspergillus niger* (3, 4). Two subgroups of dermaseptins can be identified: dermaseptins b (six members) and dermaseptins S (five members) (5). A strong synergy of action of the different dermaseptins S has been observed. Combination of the dermaseptins S causes a 100-fold increase in the efficiency of antimicrobial action (2). Shortening of the 30 amino acid dermaseptin S3 to dermaseptin S3(1–16)-NH<sub>2</sub>, does not cause a decrease in antimicrobial activity (6), although the resulting peptide is too short to span the membrane.

In the present study the mechanisms of membrane permeabilization were investigated of the designed, synthetic, antifungal peptide Ac-MB21-NH<sub>2</sub> (Ac-FASLLGKALKALAKQ-NH<sub>2</sub>) and of dermaseptin S3(1–16)-NH<sub>2</sub> (ALWKNMLKGIGKLAGK-NH<sub>2</sub>). To this end the interaction of the peptides with liposomes of different (phospho)lipid composition was studied.

## MATERIALS AND METHODS

**Materials.** Dioleoylphosphatidylglycerol (PG),<sup>1</sup> dioleoylphosphatidylcholine (PC), dioleoylphosphatidylethanolamine (PE), dioleoylglycerol, 1-palmitoyl-2-stearoyl-(5-doxyl)-sn-glycero-3-phosphocholine (abbreviated 5-doxyl-PC), and 1-palmitoyl-2-stearoyl-(16-doxyl)-sn-glycero-3-phosphocholine (abbreviated 16-doxyl-PC) were purchased from Avanti Polar Lipids Inc. (Birmingham, AL). L- $\alpha$ -Phosphatidylserine (PS) from bovine brain and ergosterol were obtained from Sigma. F1W-Ac-MB21-NH<sub>2</sub>, Ac-MB21-NH<sub>2</sub>, and dermaseptin S3(1–16)-NH<sub>2</sub> were obtained from Affinity Research Products Ltd., Exeter, EX6 8HD U.K.

**Liposome Preparation.** Phospholipids with or without ergosterol (molar ratio ergosterol/phospholipid 0.8) dissolved in organic solvent were dried under nitrogen; subsequently, a small volume of ethanol was added, and the drying step was continued. Multilamellar vesicles were formed by vortexing in the presence of buffer and glass beads. Small unilamellar vesicles were obtained by sonication (tip diameter 3 mm; Soniprep 150, MSE Ltd., Crawley, Sussex) of 500  $\mu$ L of the multilamellar vesicles (3–6 mM phospholipid),

<sup>†</sup> These investigations were supported by The Netherlands Foundation for Chemical Research (CW) with financial aid from The Netherlands Organization for Scientific Research (NWO) and Unilever Research Vlaardingen.

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<sup>1</sup> Abbreviations: PG, dioleoylphosphatidylglycerol; PC, dioleoylphosphatidylcholine; PE, dioleoylphosphatidylethanolamine; PS, L- $\alpha$ -phosphatidylserine; DOG, dioleoylglycerol; 5-doxyl-PC, 1-palmitoyl-2-stearoyl-(5-doxyl)-sn-glycero-3-phosphocholine; 16-doxyl-PC, 1-palmitoyl-2-stearoyl-(16-doxyl)-sn-glycero-3-phosphocholine; CD, circular dichroism.

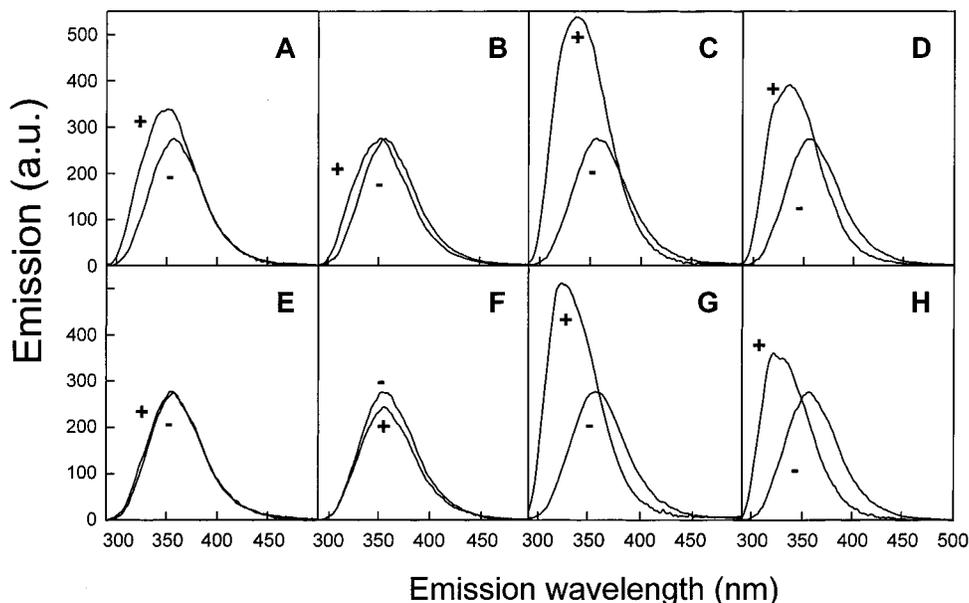


FIGURE 1: Liposome interaction of peptides measured by tryptophan fluorescence. Spectra of F1W-Ac-MB21-NH<sub>2</sub> (panels A–D) and dermaseptin S3(1–16)-NH<sub>2</sub> (panels E–H) in the presence (+) and absence (–) of liposomes. Panels: A and E, PC liposomes; B and F, ergosterol/PC liposomes; C and G, PG liposomes; and D and H, ergosterol/PG liposomes. Peptides and phospholipid vesicles were present at 2 and 50  $\mu$ M, respectively.

10  $\mu$ m amplitude, pulses of 45 s on/15 s off, incubation on ice under a stream of nitrogen, during 15–30 min until the suspension was transparent. Metal particles and large vesicles were removed by 10 min centrifugation at 5400g and 4 °C.

**Membrane Potential Measurement.** Liposomes (74  $\mu$ M final phospholipid concentration) containing 50 mM potassium phosphate, pH 7.0, were suspended in 50 mM sodium phosphate, pH 7.0, and valinomycin (250 nM) was added to induce a transmembrane potential ( $\Delta\psi$ ). The  $\Delta\psi$  was monitored by means of DiSC<sub>(3)5</sub> fluorescence (7) using excitation and emission wavelengths of 643 and 666 nm, respectively. The slit widths were set at 10 nm. Corrections were made for the peptide-induced decrease in DiSC<sub>(3)5</sub> fluorescence in the absence of a  $\Delta\psi$ .

**Measurement of Peptide Concentration.** Peptide concentrations were determined by measuring the optical density at 214 nm for MB21 ( $\epsilon = 12\,790.8\text{ cm}^{-1}\text{ M}^{-1}$ ) and at 280 nm for the tryptophan-containing peptides ( $\epsilon = 5600\text{ cm}^{-1}\text{ M}^{-1}$ ).

**Circular Dichroism.** CD spectra were recorded on an Aviv 62A DS CD spectrometer. The temperature was kept constant at 25 °C, and the sample compartment was flushed with N<sub>2</sub>. Spectra are the averages of 5–10 scans, using a band- and stepwidth of 1 nm. Experiments were performed in 50 mM potassium phosphate, pH 7.0.

**Fluorescence Measurements.** Fluorescence was measured with a Perkin-Elmer LS-50 spectrofluorometer. Tryptophan fluorescence was measured using an excitation wavelength of 280 nm with the excitation and emission slit widths set at 2.5 and 5 nm, respectively. Experiments were performed at 30 °C in 50 mM potassium phosphate, pH 7.0, unless mentioned otherwise. Spectra were corrected for volume changes, and the background with buffer or liposome suspension without peptide was subtracted. In the case of tryptophan quenching by KI, additional controls were performed with KCl instead of KI. All experiments were

repeated in three to five independent experiments. Data are presented either by a typical experiment or as the means with standard deviation.

## RESULTS

**Membrane Interaction.** The interaction of Ac-MB21-NH<sub>2</sub> and dermaseptin S3(1–16)-NH<sub>2</sub> with the phospholipid surface was followed by exploiting tryptophan fluorescence. Since Ac-MB21-NH<sub>2</sub> lacks an endogenous tryptophan residue, phenylalanine at position 1 was substituted for a tryptophan, and the resulting peptide, F1W-Ac-MB21-NH<sub>2</sub>, was obtained after solid-state synthesis. (F1W-Ac-MB21-NH<sub>2</sub> was equipotent to Ac-MB21-NH<sub>2</sub> in dissipating the  $\Delta\psi$  in target cells; data not shown).

The interaction of F1W-Ac-MB21-NH<sub>2</sub> with PC liposomes results in a blue shift of the emission maximum of about 7 nm and an increase in quantum yield (31%) (Figure 1A). The tryptophan of F1W-Ac-MB21-NH<sub>2</sub> inserted more deeply into PG than into PC liposomes, as the emission blue shift in the case of PG liposomes was much larger, i.e., 21 nm, with an almost 2-fold (88%) increase in fluorescence quantum yield (Figure 1C).

Dermaseptin S3(1–16)-NH<sub>2</sub> contains a tryptophan residue at position 3. W3 of dermaseptin shows a negligible blue shift in its fluorescence maximum (1 nm) and quantum yield (2%) upon interaction with PC liposomes (Figure 1E). In contrast, upon interaction with PG liposomes, a very large blue shift (34 nm) and increase in fluorescence quantum yield (64%) is observed (Figure 1G), which suggests that the tryptophan of this peptide selectively inserts into anionic lipids.

Insertion of the tryptophan of F1W-Ac-MB21-NH<sub>2</sub> and of dermaseptin S3(1–16)-NH<sub>2</sub> resulted in the same emission peak shift within a large range of peptide/phospholipid ratios: 1/25 to 1/750 (data not shown). This suggests that the insertion of the tryptophans does not depend on the peptide/lipid ratio.

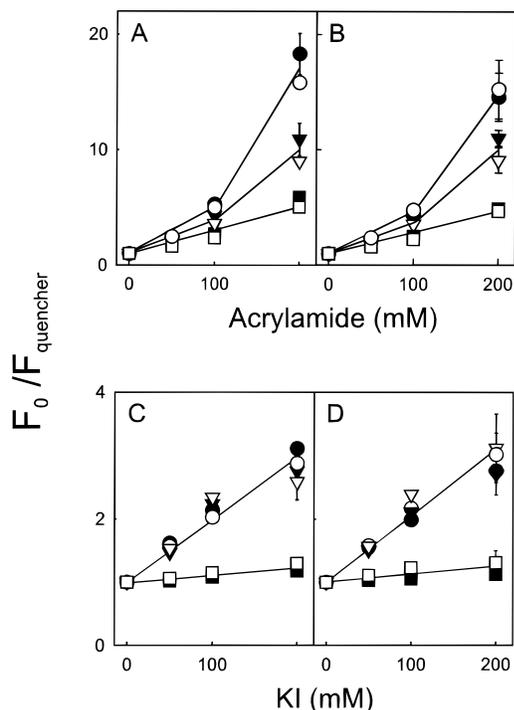


FIGURE 2: Stern–Volmer plots of quenching of tryptophan fluorescence: A and C, F1W-Ac-MB21-NH<sub>2</sub>; B and D, dermaseptin S3(1–16)-NH<sub>2</sub>. Quenching was done with acrylamide and potassium iodide without lipids (●) and in the presence of 40  $\mu$ M ergosterol suspension (○), PC liposomes (▼), ergosterol/PC liposomes (▽), PG liposomes (■), or ergosterol/PG liposomes (□). Peptides and phospholipid vesicles were present at 2 and 50  $\mu$ M, respectively.

Fungal membranes contain ergosterol. In the case of yeast membranes, ergosterol/phospholipid molar ratios can even be as high as 0.81 (8) or 0.94 (9) but not higher than 1.0 (10). The effect of ergosterol on the interaction of both amphipatic peptides with the membrane was determined. Both with F1W-Ac-MB21-NH<sub>2</sub> (Figure 1B,D) and with dermaseptin S3(1–16)-NH<sub>2</sub> (Figure 1F,H), the presence of ergosterol had little effect on the size of the induced peak blue shift. Therefore, it seems that ergosterol hardly alters the extent of peptide association and insertion into the membrane. Ergosterol, however, limited the increase in fluorescence quantum yield: compare Figure 1B,D,F,H (with ergosterol) with the corresponding Figure 1A,C,E,G, respectively (without ergosterol).

To determine the orientation of the peptides relative to the lipid surface, experiments were performed with water-soluble (acrylamide and potassium iodide) and membrane-embedded quenchers (5- and 16-doxyl-PC) of tryptophan fluorescence. The presence of PC liposomes (triangles, Figure 2A,B) slightly reduced the acrylamide quenching (compare with no liposomes, circles, Figure 2A,B) but had no effect on the potassium iodide quenching (Figure 2C,D). This indicates that the tryptophan residues do not insert deeply into the PC membranes. In contrast, with PG liposomes, the effectiveness of acrylamide and KI was markedly reduced (squares, Figure 2A–D). This suggests that the tryptophan residues of F1W-Ac-MB21-NH<sub>2</sub> and dermaseptin S3(1–16)-NH<sub>2</sub> insert into the anionic membranes, thereby becoming less accessible to the aqueous quenchers. The presence of ergosterol (open symbols, Figure 2A–D) either in suspension or incorporated into the liposomes did not affect the

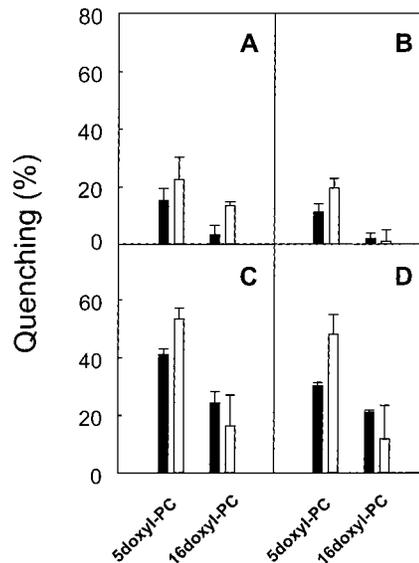


FIGURE 3: Quenching of tryptophan fluorescence by doxyl-PC: A and C, F1W-Ac-MB21-NH<sub>2</sub>; B and D, dermaseptin S3(1–16)-NH<sub>2</sub>. PC liposomes (A and B; 50  $\mu$ M phospholipid) and PG liposomes (C and D; 50  $\mu$ M phospholipid) with or without 10% of 5- or 16-doxyl-PC were added to a solution containing 2  $\mu$ M F1W-Ac-MB21-NH<sub>2</sub> or dermaseptin S3(1–16)-NH<sub>2</sub>. The tryptophan fluorescence spectra were recorded before and after addition. Bars: solid, liposomes without ergosterol; open, liposomes of ergosterol/phospholipid.

efficiency of quenching (compare with the corresponding closed symbols).

To assay the depth of penetration, the quenchers 5- and 16-doxyl-PC were used in experiments with F1W-Ac-MB21-NH<sub>2</sub> (Figure 3A,C) and dermaseptin S3(1–16)-NH<sub>2</sub> (Figure 3B,D). The quenching groups of these molecules are at different depths (positions 5 and 16 of the acyl chains), and they are present in both leaflets of the liposome membrane. Quenching by the doxyl-PC molecules was much stronger when the peptides were bound to PG liposomes (Figure 3C,D) as compared to PC liposomes (Figure 3A,B). In all cases, 5-doxyl-PC was more effective in quenching than 16-doxyl-PC. The presence of ergosterol in the liposomes (gray bars) had no major effect on the extent of quenching. Taken together, these data suggest that the amino termini of Ac-MB21-NH<sub>2</sub> and dermaseptin S3(1–16)-NH<sub>2</sub> penetrate into the acyl chain region of anionic phospholipid membranes.

**Lipid-Induced Secondary Structure.** Circular dichroism was used to analyze the effect of the liposomes on the secondary structure of the peptides. In aqueous solution, Ac-MB21-NH<sub>2</sub> (Figure 4A) and dermaseptin S3(1–16)-NH<sub>2</sub> (Figure 4B) are present in random coil structure (Figure 4, curves a). Some secondary structure was elicited in Ac-MB21-NH<sub>2</sub> (possibly  $\beta$ -sheet or a mixture of  $\beta$ -sheet and  $\alpha$ -helix) by liposomes composed of PC (Figure 4A, curve c) or PC/ergosterol (Figure 4A, curve b). On the other hand, neither PC liposomes nor PC/ergosterol liposomes had an effect on the structure of dermaseptin S3(1–16)-NH<sub>2</sub> (Figure 4B, curves c and b, respectively). By contrast, PG (Figure 4A,B, curve e) and PG/ergosterol (Figure 4A,B, curve d) liposomes clearly induce negative ellipticity at 208 and 222 nm with both peptides, which is characteristic for  $\alpha$ -helix formation.

*Ac-MB21-NH<sub>2</sub> and Dermaseptin S3(1–16) Dissipate  $\Delta\psi$  in Liposomes.* Membrane permeabilization by Ac-MB21-

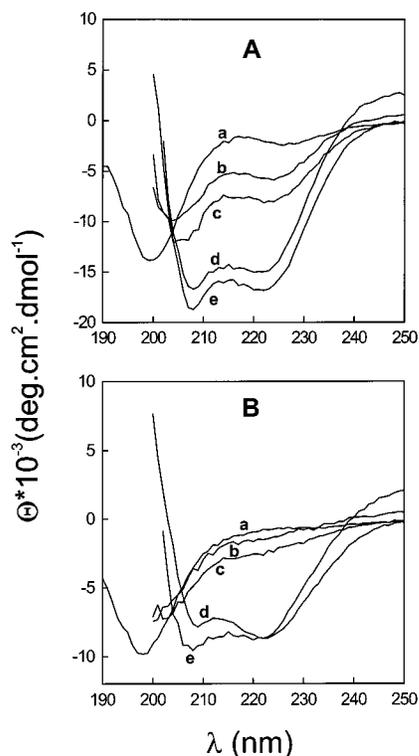


FIGURE 4: Lipid-induced secondary structure: A, Ac-MB21-NH<sub>2</sub> (98  $\mu$ M), and B, dermaseptin S3(1-16)-NH<sub>2</sub> (95  $\mu$ M). Circular dichroism spectra without liposomes (a) and in the presence of ergosterol/PC liposomes (b), PC liposomes (c), ergosterol/PG liposomes (d), or PG liposomes (e) are shown. The phospholipid concentration of the liposome suspensions was 2 mM.

NH<sub>2</sub>, F1W-Ac-MB21-NH<sub>2</sub>, and dermaseptin S3(1-16)-NH<sub>2</sub> was followed by measuring their ability to dissipate a valinomycin-induced  $\Delta\psi$  in liposomes (Figure 5). In the case of PC liposomes, Ac-MB21-NH<sub>2</sub> (Figure 5A) and F1W-Ac-MB21-NH<sub>2</sub> (Figure 5B) showed a low  $\Delta\psi$  dissipating activity. In contrast, dermaseptin S3(1-16)-NH<sub>2</sub> (Figure 5C) was completely ineffective. All three peptides more efficiently dissipated the  $\Delta\psi$  in anionic PG liposomes. Ac-MB21-NH<sub>2</sub> and F1W-Ac-MB21-NH<sub>2</sub> had identical activity, while dermaseptin S3(1-16)-NH<sub>2</sub> appeared less effective. The presence of ergosterol had little effect on the effectiveness of these peptides.

The capacity of Ac-MB21-NH<sub>2</sub> (Figure 6A) and dermaseptin S3(1-16)-NH<sub>2</sub> (Figure 6B) to dissipate the  $\Delta\psi$  in liposomes of varying composition was further investigated. Since dermaseptin S3(1-16)-NH<sub>2</sub> was less effective than Ac-MB21-NH<sub>2</sub>, a nearly 6-fold higher concentration of dermaseptin S3(1-16)-NH<sub>2</sub> was used. The rate of  $\Delta\psi$  dissipation was affected by the lipid composition as follows: the presence of 50% (w/w) of a conic, zwitterionic (PE) or 10% of a conic, neutral [dioleoylglycerol (DOG)] lipid reduced the rate of  $\Delta\psi$  dissipation, while a cylindrical, anionic lipid (PG) enhances this rate. The presence of 50% (w/w) of the anionic PS enhanced the dissipation rate only slightly compared to pure PC liposomes. When its charge is neutralized, PS behaves like a conic lipid. For Ac-MB21-NH<sub>2</sub> (Figure 6A) PS reduced the dissipation rate compared to pure PG, but for dermaseptin S3(1-16)-NH<sub>2</sub> (Figure 6B) no significant effect was observed. The inhibitory effect of (potentially) conic lipids (PS, PE, DOG) is stronger in the case of Ac-MB21-NH<sub>2</sub> than in the case of dermaseptin S3-

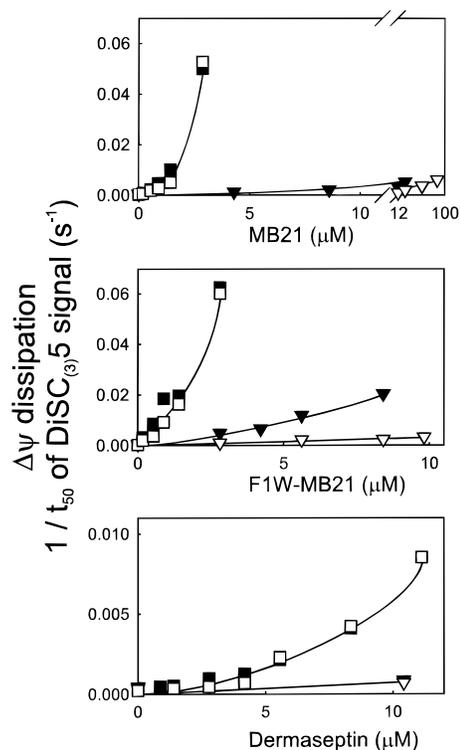


FIGURE 5: Dissipation of a valinomycin-induced  $\Delta\psi$  in liposomes: A, Ac-MB21-NH<sub>2</sub>; B, F1W-Ac-MB21-NH<sub>2</sub>; C, dermaseptin S3(1-16)-NH<sub>2</sub>. Liposomes (74  $\mu$ M phospholipid concentration after dilution) prepared with 50 mM potassium phosphate, pH 7.0, were diluted 72-fold into 50 mM sodium phosphate, pH 7.0. After addition of valinomycin (250 nM) the development of a  $\Delta\psi$  was monitored by the decrease in DiSC<sub>(3)5</sub> fluorescence. Next, peptide was added, and the time needed to depolarize the  $\Delta\psi$  for 50% was determined for PG liposomes (■), ergosterol/PG liposomes (□), PC liposomes (▼), and ergosterol/PC liposomes (▽).

(1-16)-NH<sub>2</sub>. CD measurements with each peptide showed identical peaks at 222 nm when PG, PG/PE, and PG/PC liposomes were compared (data not shown). This suggests that the percentage of  $\alpha$ -helix of each peptide is the same in these cases. Therefore, it is unlikely that the lower  $\Delta\psi$  dissipating activity of Ac-MB21-NH<sub>2</sub> in PG/PE liposomes compared to PG liposomes would be a consequence of the peptide structure.

## DISCUSSION

Herein we report on the liposome interactions of the designed Ac-MB21-NH<sub>2</sub> peptide and dermaseptin S3(1-16)-NH<sub>2</sub>. The aim of this study is to understand the broad target specificity of these peptides in terms of the phospholipid selectivity.

We show for Ac-MB21-NH<sub>2</sub> and dermaseptin S3(1-16)-NH<sub>2</sub> that the ability to dissipate the  $\Delta\psi$  correlates with the capacity to insert into a membrane and the formation of an  $\alpha$ -helical structure. Previously, an  $\alpha$ -helical structure formation has also been observed for the related dermaseptin S1(1-18)-NH<sub>2</sub> (11).

The presence of anionic phospholipids seems to enhance lipid interaction in our experiments, suggesting that a strong electrostatic interaction of these cationic peptides with the negatively charged lipid surface is a requirement for activity. These lipids are abundantly present in the outer leaflet of the cytoplasmic membrane of bacteria, explaining the high

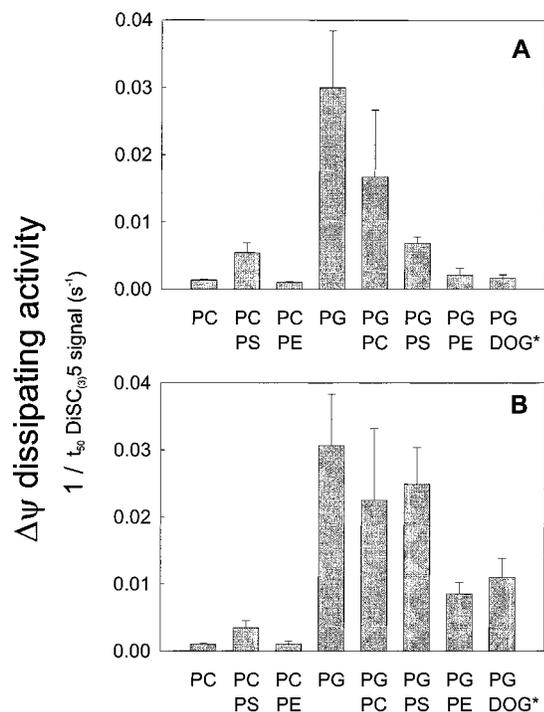


FIGURE 6:  $\Delta\psi$  dissipating activity of peptides: A, Ac-MB21-NH<sub>2</sub>, and B, dermaseptin S3(1-16)-NH<sub>2</sub>. Liposomes (74  $\mu$ M phospholipid concentration after dilution) prepared with 50 mM potassium phosphate, pH 7.0, were diluted 72-fold into 50 mM sodium phosphate, pH 7.0. After addition of valinomycin (250 nM) the development of a  $\Delta\psi$  was monitored by the decrease in DiSC<sub>(3)5</sub> fluorescence. Next, Ac-MB21-NH<sub>2</sub> (1.5  $\mu$ M) or dermaseptin S3(1-16)-NH<sub>2</sub> (8.8  $\mu$ M) was added, and the time needed to depolarize the  $\Delta\psi$  for 50% was determined. Liposomes were composed of the following: PC; PC/PS, 1/1 w/w; PC/PE, 1/1 w/w; PG; PG/PC, 1/1 w/w; PG/PS, 1/1 w/w; PG/PE, 1/1 w/w; PG/DOG, 9/1 w/w.

activity against these organisms. In yeast plasma membranes, about 25–30% of the anionic lipids are present in the outer leaflet (12). Mammalian cells generally do not expose anionic lipids in the outer leaflet of their plasma membrane and are not or hardly susceptible to cationic peptides. As a consequence Ac-MB21-NH<sub>2</sub> and dermaseptin S3(1-16)-NH<sub>2</sub> are not hemolytic (2, 13).

Ergosterol that is present at high concentration in fungal and yeast plasma membranes hardly influenced the interaction of Ac-MB21-NH<sub>2</sub> and dermaseptin S3(1-6)-NH<sub>2</sub> with the liposomes. Ergosterol, however, further reduced the poor ability of these peptides to dissipate the  $\Delta\psi$  in liposomes composed of PC, but not of PG. Sterols have been suggested to protect membranes to some extent against membrane active peptides (14, 15).

In contrast to anionic liposomes, zwitterionic PC liposomes induce no significant structure in dermaseptin, but with Ac-MB21-NH<sub>2</sub>,  $\beta$ -sheet or a mixture of  $\beta$ -sheet and  $\alpha$ -helix is formed. Strikingly, Ac-MB21-NH<sub>2</sub> seems to interact and penetrate PC liposomes, whereas this interaction is not apparent with dermaseptin.

Our quenching studies of inserted Trp residues indicate a surface interaction without deep penetration. In the case of  $\alpha$ -helical dermaseptin S3(1-16)-NH<sub>2</sub>, the large tryptophan-3 blue shift results possibly partly from the neighboring hydrophobic isoleucine (Figure 7). Some data are available on the self-association of the dermaseptins S1, S3, S4, b, and B, the precursor of b (4, 16, 17). Our data indicate that

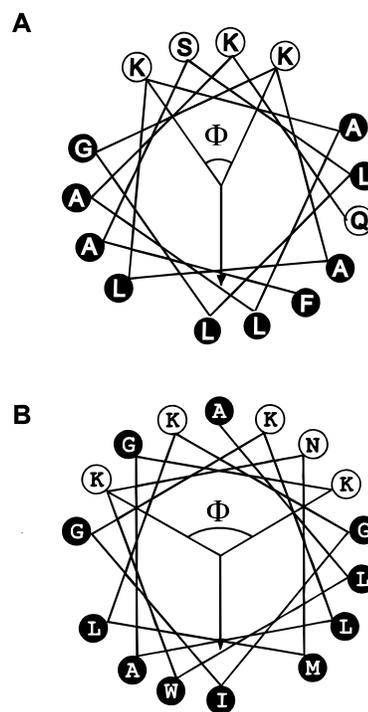


FIGURE 7:  $\alpha$ -Helical wheel presentation of Ac-MB21-NH<sub>2</sub> and dermaseptin S3(1-16)-NH<sub>2</sub>. Vectors represent the hydrophobic moments: 0.54 for Ac-MB21-NH<sub>2</sub> (A) and 0.51 for dermaseptin S3(1-16)-NH<sub>2</sub> (B) Symbols: closed, hydrophobic residues; open, hydrophilic residues.  $\Phi$  is the angle subtended by the positive residues that border the hydrophobic part of the helix. Amino acid hydrophobicity values were from Eisenberg (31).

the association of Ac-MB21-NH<sub>2</sub> and dermaseptin S3(1-16)-NH<sub>2</sub> with anionic lipids correlates with activity, but it is not clear if the molecules are present in an aggregated state.

Little is known about how these peptides permeabilize membranes. Mor and Nicolas (11) suggested a barrel stave-like pore model for dermaseptin S1. Ac-MB21-NH<sub>2</sub> and dermaseptin S3(1-16)-NH<sub>2</sub> might be too short to form membrane-spanning barrel stave pores. Shai and co-workers, however, have proposed a carpet-like mechanism for these peptides (18, 19). This model suggests that the membrane is disrupted when a threshold concentration of in-plane bound peptide is reached (20). Such a mechanism seems possible for dermaseptin because of the relatively high dermaseptin/liposome ratio. In our experiments, the effective peptide/PG ratio ranged from about 0.002 to 0.04 for Ac-MB21-NH<sub>2</sub> and from 0.01 to 0.14 for dermaseptin S3(1-16)-NH<sub>2</sub>. At a surface area of 0.716 nm<sup>2</sup>/PG molecule (21) and an average vesicle diameter of 50 nm, these ratios would correspond to about 25–422 molecules of Ac-MB21-NH<sub>2</sub>/vesicle and 127–1566 molecules of dermaseptin S3(1-16)-NH<sub>2</sub>/vesicle.

Three different models share local fusion of the membrane leaflets: the wedge-like model (22, 23), the toroidal model (24, 25), and the in-plane diffusion model (26). Another alternative model implies that wedge- or inverted wedge-shaped peptides insert in-plane into the outer leaflet, causing a disturbed lipid organization of the outer leaflet (27). The effects of insertion of “inverted conic peptides” (27, 28) might be identical to the effects of insertion of peptides with a small angle  $\Phi$ , the angle subtended by the charged residues

(Figure 7). With a decrease in the angle  $\Phi$ , the higher the activity would be (29). Strikingly,  $\Phi$  of the more active (Figure 5) Ac-MB21-NH<sub>2</sub> is 60°, which is two times smaller than  $\Phi$  of dermaseptin S3(1-16)-NH<sub>2</sub> (Figure 7). Figure 6 shows that conic-shaped lipids are more strongly inhibiting Ac-MB21-NH<sub>2</sub>, which has a smaller  $\Phi$  than dermaseptin S3(1-16)-NH<sub>2</sub>. For both peptides PS is less stimulatory than PG. This was also observed in the case of magainin (30) and might relate to the conic shape that PS may adopt when interacting with a cationic peptide.

Taken together, the present data on Ac-MB21-NH<sub>2</sub> and dermaseptin S3(1-16)-NH<sub>2</sub> suggest that the  $\alpha$ -helicity of the peptides, the angle  $\Phi$ , subtended by the charged residues in  $\alpha$ -helical peptides, and the shape and charge of the phospholipids in the target membrane are important factors in determining the capacity of the peptides to permeabilize the membrane. The small angle  $\Phi$  in  $\alpha$ -helical Ac-MB21-NH<sub>2</sub>, as compared to  $\Phi$  in dermaseptin S3(1-16)-NH<sub>2</sub>, might be responsible for its higher capacity to disturb the lipid bilayer organization. Conic lipids strongly counteract the strongly membrane disturbing Ac-MB21-NH<sub>2</sub> but counteract to a lesser extent dermaseptin S3(1-16)-NH<sub>2</sub>.

#### ACKNOWLEDGMENT

Shanti Dielbandhousing is gratefully acknowledged for assistance and Marcel L. de Vocht for help with circular dichroism measurements. Herman Berendsen, Allen Mark, and Peter Tieleman are acknowledged for stimulating discussions.

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BI000917A