Membrane Interactions of Antimicrobial β-Peptides: The Role of Amphipathicity Versus Secondary Structure Induction

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ABSTRACT:

The membrane interaction of two beta peptides was studied using a surface plasmon resonance biosensor. The two peptides are β-17, a novel β-amino acid based antimicrobial peptide and the corresponding scrambled-β17—a non-antimicrobial β-peptide analogue. Membrane interaction studies were performed with a series of phospholipid mixtures which mimic either mammalian cells (high in phosphatidylcholine and cholesterol) or microbial cells (high in phosphatidylethanolamine and phosphatidylglycerol). The results were compared with the membrane binding of the well-characterized antimicrobial peptide magainin 2. The secondary structure of these peptides were also determined in each lipid mixture by circular dichroism and correlated with the membrane-binding properties. Both β-17 and the scrambled peptide have the same peptide length, charge and showed a similar secondary structure in both aqueous buffer and in the presence of liposomes. Both peptides also bound to a similar level on each membrane mixture, showing that the dramatic difference in biological activity is not based on the amount of peptide bound but rather differences in the degree of insertion and rate of membrane dissociation. Although β-17 and the scrambled β-17 peptide exhibited similar binding properties on all membrane mimics, both β-peptides bound more to all membranes compared with magainin 2. Overall, the results further reveal the significant interplay between peptide amphipathicity and secondary structure induction on membrane binding.

Keywords: surface plasmon resonance; peptide-lipid interactions; antimicrobial peptide; beta peptide; phospholipid membrane

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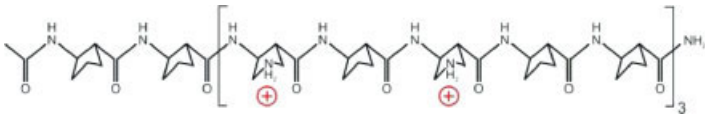
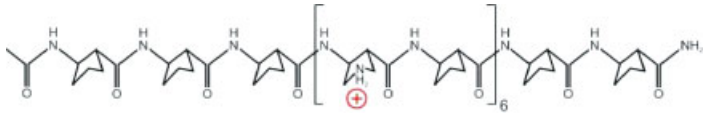
INTRODUCTION

Increasing resistance of microbes to conventional antibiotics has sparked renewed interest in antimicrobial peptides as a viable alternative over recent years. Antimicrobial peptide action is usually mediated by a direct interaction with cell membranes.[1] A common feature of these interactions is the induction of cationic amphipathic secondary structure following binding of the peptides to the membrane surface. However, one of the challenges of designing peptide-based antibiotics is that control of their secondary structures is limited and they are prone to enzymatic degradation before they can achieve their effect. This has stimulated research into β-peptides as an alternative template for antimicrobial peptide design.[2,3] β-Peptides are comprised of β-amino acids which are similar to their α-amino acid counterparts but contain an additional carbon atom between the amino and carboxyl terminus. β-Amino acids with a specific side chain can exist at the α-carbon (C2) or the β-carbon

Table I Sequences of the β17 and Scrambled Peptide Containing the β-Amino Acids APC and ACPC[14] and that of Magainin 2

Peptide	Amino Acid Sequence	M_r	AA	Charge
β-17		1879	17	+6
Scrambled β-17		1879	17	+6
Magainin 2	GIGKFLHSAKKFGKAFVGEIMNS	2467	23	+5

(C3) in either the S or R isomer form and these combinations give rise to a total of four possible diastereoisomers for any given side chain carbon.[4] The versatility of being able to manipulate substituents around the C2 and C3 bonds has proven to be very attractive for molecular design, producing a range of peptides that can adopt different stable helical conformations[5] along with turn, β-hairpin and sheet conformations that have all been discovered over the past decade.[6–8] There are more opportunities for control of structural propensities with β-amino acids than with α-amino acids, as each β-amino acid can be incorporated into a ring which reduces residue flexibility without destroying the backbone hydrogen binding sites.[5,9,10] This is not possible with α-amino acid backbones, as incorporating a ring (such as proline) eliminates hydrogen bond donor sites.[11] Given this choice of secondary structures[7,8,12] and the fact that β-peptides are easy to synthesize and resistant to protease degradation,[13] it is likely that β-peptides will provide promising antimicrobial candidates in the years to come.

β-17 is a β-peptide that mimics the cationic α-helical antimicrobial peptides such as magainin.[2] β-17 consists of 17 residues and is made up of a combination of two β-amino acids: (3R,4S)-*trans*-4-aminopyrrolidine-3-carboxylic acid (APC), and (R,R)-*trans*-2-aminocyclopentanecarboxylic acid (ACPC) (Table I). The combination of these two β-amino acid residues forms what is known as a 12-helix conformation,[14] which is defined by 12-membered ring hydrogen bonds between each backbone carbonyl group and the amide NH of the third residue in the carboxy-terminal direction.[5] With two and a half residues per turn, the conformation of this 12-helix is amphipathic, in which the cationic APC residues are aligned along one side of the 12-helix and the hydrophobic ACPC residues along the other side (Figure 1). β-17 has shown strong antimicrobial activity[2,14] including activity against vancomycin resistant *Enterococcus faecium* A436 and methicillin resistant *Staphylococcua aureus* 5332 (MRSA 5332).

To assess whether the cytolytic activity of β-17 was due to its amphipathic structure, a "scrambled" peptide (scrambled β-17) was also created.[14] This peptide contains the same number and ratio of ACP and ACPC residues as β-17, but was designed with the aim of distributing the positive and hydrophobic residues around the helix in a random order, hence removing the amphipathic structure. Scrambled β-17 showed little or no activity against the same species that β-17 was shown to be active against.[2,14] This supports the hypothesis that this class of β-peptides must be amphipathic to elicit antimicrobial activity. β-17 was also found to have lower hemolytic activity than (Ala[8,13,18])-magainin 2-amide.[2] Significant hemolysis did not occur until concentrations above that of the MIC indicating that β-17 has significant selectivity for microbial cells. The scrambled peptide showed no hemolytic activity in the same concentration range.[2]

Since selective binding to different phospholipids is central to the design of nonhemolytic antimicrobial peptides, the affinity of the peptide for the membrane surface is a critical factor in the cell-lytic process. This study investigates the interaction of β-17, the scrambled β-17 analogue, and magainin 2 with four different membrane mixtures by circular dichroism (CD) and surface plasmon resonance (SPR) and provides insight into the structure-activity relationships of this novel class of antimicrobial peptides.

MATERIALS AND METHODS

Chemicals and Reagents

Sodium phosphate dibasic/monobasic, (3-cholamidopropyl)-dimethylammonio]-1-propanesulfonate) (CHAPS), 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine (DMPC), 1,2-dimyristoyl-*sn*-glycero-

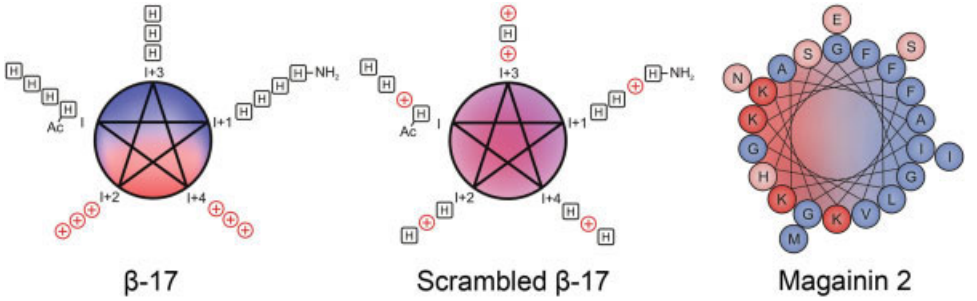


FIGURE 1 Helical diagram of β-17 and scrambled β-17 showing the 2.5 residue repeat 12-helix. The peptides amphipathic structure is highlighted by one side of the helix being cationic (red area) and the other side hydrophobic (blue area).[14] The scrambled β-17 peptide has the positively charged and hydrophobic residues randomly distributed around the helix so the peptide is not amphipathic.[14]

3-[phospho-*rac*-(1-glycerol)] (sodium salt) (DMPG), 1,2-dimyristoyl-*sn*-glycero-3-phosphoethanolamine (DMPE), cholesterol, and magainin 2 were purchased from Sigma (St Louis, MO). Sodium chloride was purchased from Spectrum Chemical (Gardena, CA). Chloroform and methanol were purchased from Merck (Victoria, Australia), water was quartz-distilled and deionized in a milli-Q system (Millipore, Bedford, MA). β-17 and scrambled β-17 were synthesized as previously described by Dr. Naoki Umezawa and Dr. Emilie Porter[5] and were generously provided by Professor Samuel Gellman, (University of Wisconsin).

Liposome Preparation

DMPC and cholesterol were dissolved in chloroform, DMPG was dissolved in a mixture of chloroform/methanol (3:1 v/v) and DMPE was dissolved in a mixture of chloroform/methanol (1:1 v/v) to create individual stock solutions. These stock solutions were then aliquoted into test tubes in the desired ratios: DMPC, DMPC/PG (4:1 v/v), DMPC/PG/cholesterol (16:4:5 v/v), DMPE/PG (4:1 v/v). The solvent was then evaporated under a gentle stream of N_2 and vacuum desiccated overnight. For CD experiments, lipids were resuspended in 20 m*M* phosphate buffer pH 6.8 with vortexing to a concentration of 1.36 m*M*. The resultant lipid dispersion was then sonicated with a bath type sonicator until clear. For SPR experiments, lipids were resuspended into buffer with vortexing to a concentration of 0.5–1 m*M*. The lipid dispersion was then sonicated in a bath type sonicator until nearly clear. Small unilamellar vesicles (SUV; 50 nm) were created via extruding the sonicated mixture through a 50 nm polycarbonate membrane filter (Avestin, Ottawa, ON).

Circular Dichroism

CD measurements were carried out on a Jasco J-810 CD spectropolariser (Jasco, Tokyo, Japan). Using quartz cells of 1 mm path length, scans between wavelengths of 190 and 260 nm were done with a scan speed of 20 nm/min and a bandwidth of 1.0 nm. The resolution was 0.1 nm with a 1 s response time, five scan accumulations. The quartz cell temperature was controlled with a peltier temperature controller at 25°C. The CD instrument was calibrated with (+)-10-camphorsulphonic acid. The different lipid liposomes were prepared as mentioned earlier. The concentration of these lipids was 1.36 m*M*. Once the lipid solution was ready, peptide was then added to a peptide lipid ratio of ~ 1:100. This was then sonicated briefly just before measurements. The same peptide concentration was used for samples with peptide in buffer solution alone. The CD spectra were measured for the peptides in phosphate buffer solution and in the presence of the different lipid liposomes. The final spectra obtained for each peptide is the average of five accumulated scans. Spectra were smoothed using the Jasco Fast Fourier transform algorithm and baseline corrected. Following baseline correction, the percentage of helix was calculated for the peptide in buffer and in the presence of the different lipid solutions. This was calculated from the mean residue ellipticity $[\theta]$ at 222 nm (deg cm^2 dmole^{-1}) according to the relationship as follows[15]:

$$\%\,\alpha = 100 * [\theta]_{222}/\theta_f \text{ and } \theta_f = -39500 * (1 - 2.57/n)$$

where α is the amount of helix

Surface Plasmon Resonance

SPR experiments were carried out with a Biacore 3000 analytical system with an L1 sensor chip [GE-Healthcare (Biacore), Uppsala, Sweden]. The L1 chip surface contains lipophilic groups which are covalently attached to carboxymethylated dextran. This allows direct attachment of lipid membrane vesicles such as liposomes. The lipid bilayer structure is retained upon attachment, facilitating the study of peptide-membrane interactions.[16,17] The system was cleaned using the "desorb and sanitize" protocol with a maintenance chip and then allowed to run overnight with water. The L1 chip was docked and first washed with an injection of 5 μl of 20 m*M* CHAPS at a flow rate of 5 μl/min to clean the chip surface. SUVs in immobilization buffer (20 m*M* phosphate buffer 150 m*M* NaCl pH 6.8) were then immediately applied to the chip surface with injections of 80 μl at a low flow rate of 2 μl/min. To remove any mutilamellar structures from the lipid surface and to stabilize the baseline, 30 μl of 10 m*M* NaOH was injected at 50 μl/min (as recommended by the manufacturer). All solutions were freshly prepared, degassed and filtered through a 0.2 μm filter.

The peptide solutions were prepared by dissolving β-17, scrambled β-17, and magainin 2 in the running buffer (20 m*M* phos-

phate buffer pH 6.8) creating eight serial dilutions of 0.5 to 10 μM. A total of 100 μl of these solutions were injected at a flow rate of 30 μl/min having a total injection time of 200 s. On completion of injection, buffer flow continued to allow a dissociation time of at least 600 s. All binding experiments were carried out at 25°C. The affinity of the antimicrobial peptide-lipid binding event was determined from analysis of a series of response curves in each case, where the resultant sensorgrams were collected from peptide injections at different concentrations over each different lipid surface. Kinetic analysis of the sensorgrams was performed using the langmuir, the parallel and the two state curve fitting models as described in previous investigations for other peptide-membrane interactions.[16–19]

RESULTS

Peptide Secondary Structure Measured by CD

The secondary structure of β-17 and scrambled β-17 were investigated by CD in aqueous buffer and in the presence of DMPC, DMPC/DMPG (4:1), DMPC/DMPG/cholesterol (16:4:5), and DMPE/DMPG (4:1) liposomes. Figure 2a shows the CD spectra for β-17 in buffer and in the presence of the different liposome solutions. Similar spectra were observed for β-17 in all solutions but the spectra are different to that observed for a typical α-helix. Specifically, each spectrum shows a maximum and minimum in molar ellipticity in both the buffer and in the presence of the different lipid solutions, with a maximum around 203–207 nm and minimum around 225 nm. This is typical of a 12-helix and is similar to previously published CD spectra of β-peptides.[20] The signal was slightly higher in the presence of the lipids compared with the buffer, and was very strong in the presence of DMPC/DMPG (4:1) and slightly less in DMPE/DMPG (4:1).

Figure 2b shows the CD spectra for scrambled β-17 in buffer and in the presence of the different liposomes. The maxima $[\theta]$ are similar to those seen with β-17 at around 203–207 nm and minima $[\theta]$ at around 225 nm. Again the maximum $[\theta]$ for the scrambled peptide in buffer is at a shorter wavelength and the peptide shows greater helical content in the presence of liposomes compared with the buffer alone. There was again less structure in the presence of DMPE/DMPG (4:1). Overall, the scrambled peptide shows a greater helical content compared with β-17.

Figure 2c shows the CD spectra of magainin 2 in buffer and in the presence of the different lipid liposomes. Magainin 2 showed no structure in buffer solution or in the presence of DMPC alone. However, magainin 2 adopted 40% helix in DMPC/DMPG, 25% helix in DMPC/DMPG/cholesterol, and 32% helix in DMPE/DMPG. The lack of structure in aqueous buffer is consistent with previous studies that have shown that magainin 2 requires the presence of organic solvent to adopt secondary structure.[21–23] It is unclear why no structure was seen in the presence of DMPC as magainin 2 has been previously shown to adopt helical structure in the presence of egg-PC liposomes.[23] A number of experimental parameters may have contributed to the difference in the CD spectra for magainin 2 in the presence of DMPC liposomes. Egg PC contains a mixture of different acyl chains including the saturated and unsaturated species (see Avanti Polar Lipids: http://www.avantilipids.com). In addition, we used lower peptide and lipid concentrations,

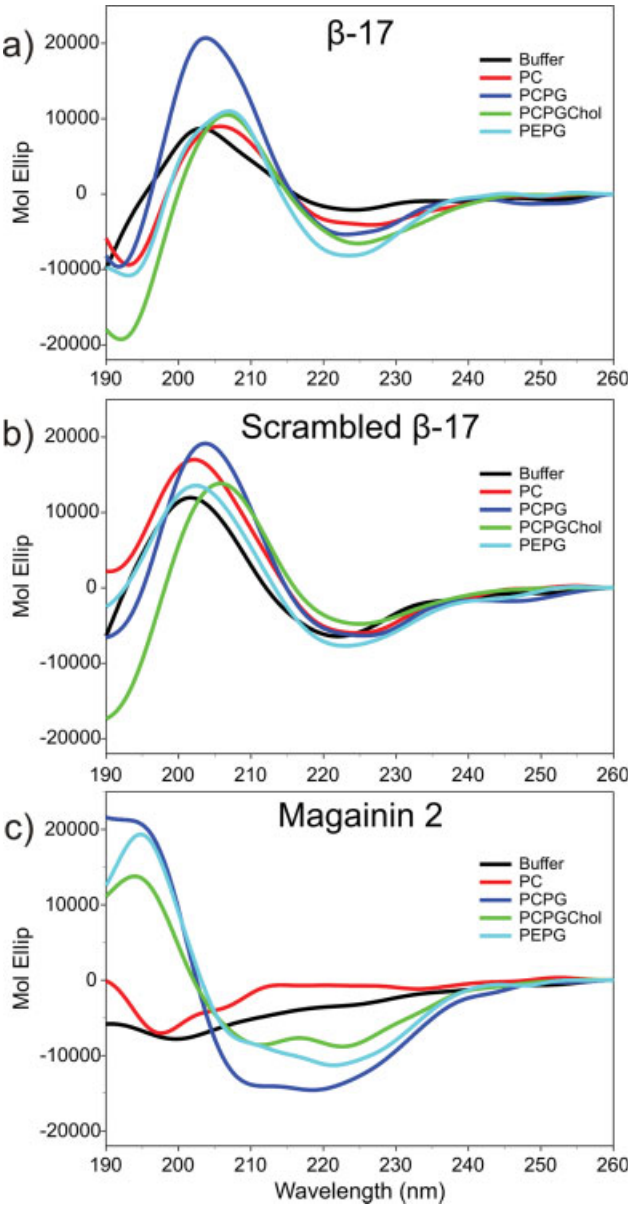


FIGURE 2 The CD spectra of each peptide in buffer and with the different liposomes: (a) β-17, (b) scrambled β-17, (c) magainin 2. 13.6 μM of peptide was used with a peptide to lipid ratio of 1:100.

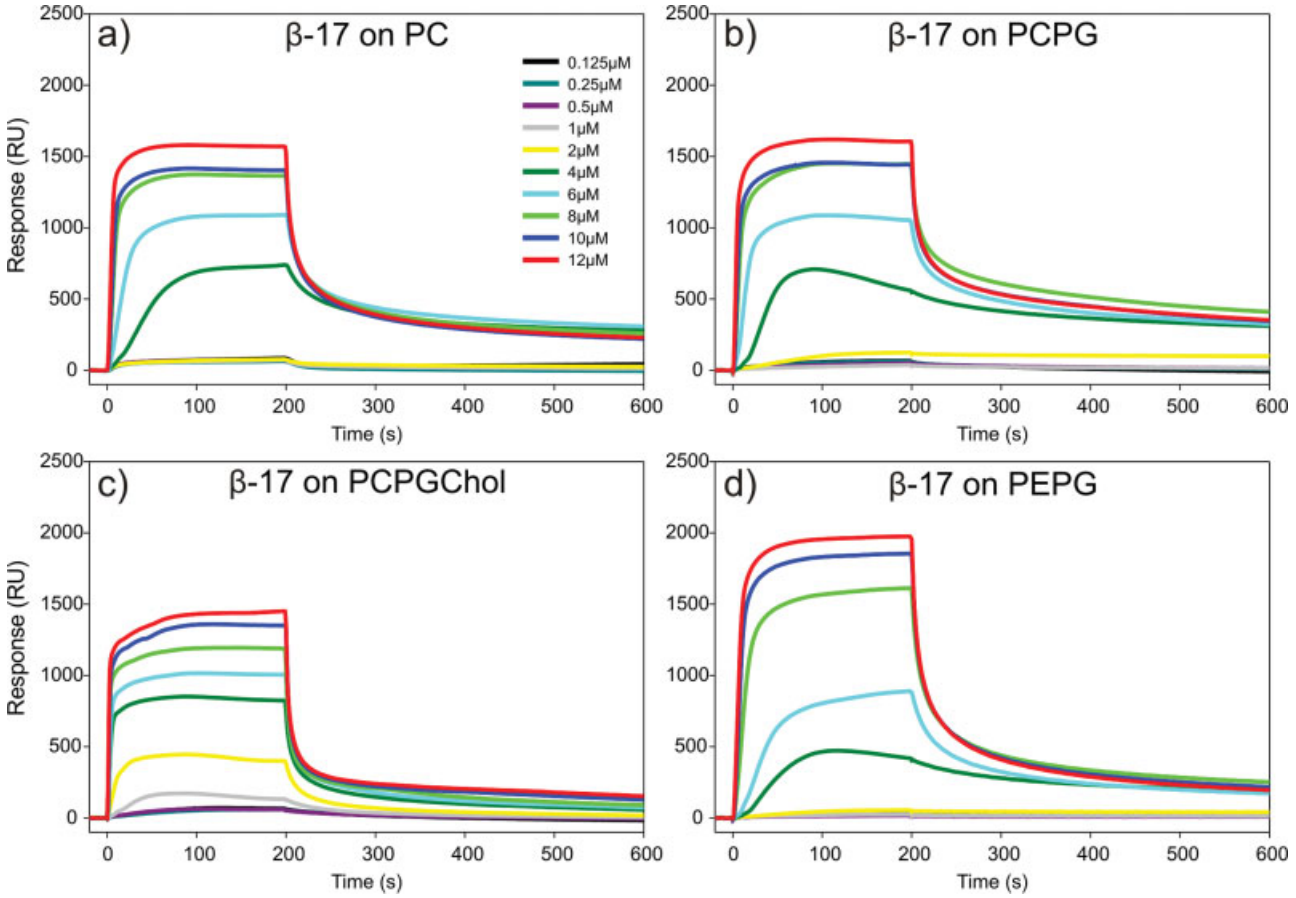


FIGURE 3 SPR sensorgrams of β-17 on the different lipid layer mixtures: (a) DMPC, (b) DMPC/DMPG (4:1), (c) DMPC/DMPG/cholesterol (16:4:5), (d) DMPE/DMPG (4:1) at 10 different concentrations from 0.125 to 12 μM. Injections were 200 s ($t = 0$–200 s) at 30 μl/min and the peptide was then allowed to dissociate for a further 400 s ($t = 200$–600 s) as buffer continued to flow through the system.

and a peptide:lipid ratio of 1:100 (peptide = 13.6 μM, lipid 1.36 mM), whereas the previous study used 100 μM peptide and 5 mM lipid (peptide:lipid ratio of 1:50). It is possible that the previous study observed concentration-dependent aggregation and induction of helical conformation and/or magainin 2 has a lower solubility in synthetic DMPC which prevented significant lipid interaction and yielded poor spectra.

Membrane Binding by SPR

The membrane binding properties of β-17, scrambled β-17, and magainin 2 were investigated by SPR on DMPC, DMPC/DMPG (4:1), DMPC/DMPG/cholesterol (16:4:5), and DMPE/DMPG (4:1) membrane mixtures. Sensorgrams were obtained at 10 different concentrations from 0.125 to 12 μM. The DMPC-containing membranes represent mimics of mammalian cell membranes while the DMPE-containing membranes were used as mimics of bacterial cell membranes.

β-17

Figure 3 shows typical sensorgrams obtained for the binding of β-17 to the different lipid compositions. On DMPC, the rate at which β-17 binds to the lipid was dependant on concentration as there was a faster association rate with the higher concentrations (4–12 μM) than with the lower concentrations (0.5–2 μM). The lowest concentrations of 0.125 and 0.25 μM showed very little binding to the membrane. There was a clear gap in response between those and the higher concentrations (4–12 μM) that showed a fast increase in response that eventually leveled to equilibrium by the end of the injection. The highest concentration of peptide (12 μM) bound very quickly to reach a maximum response

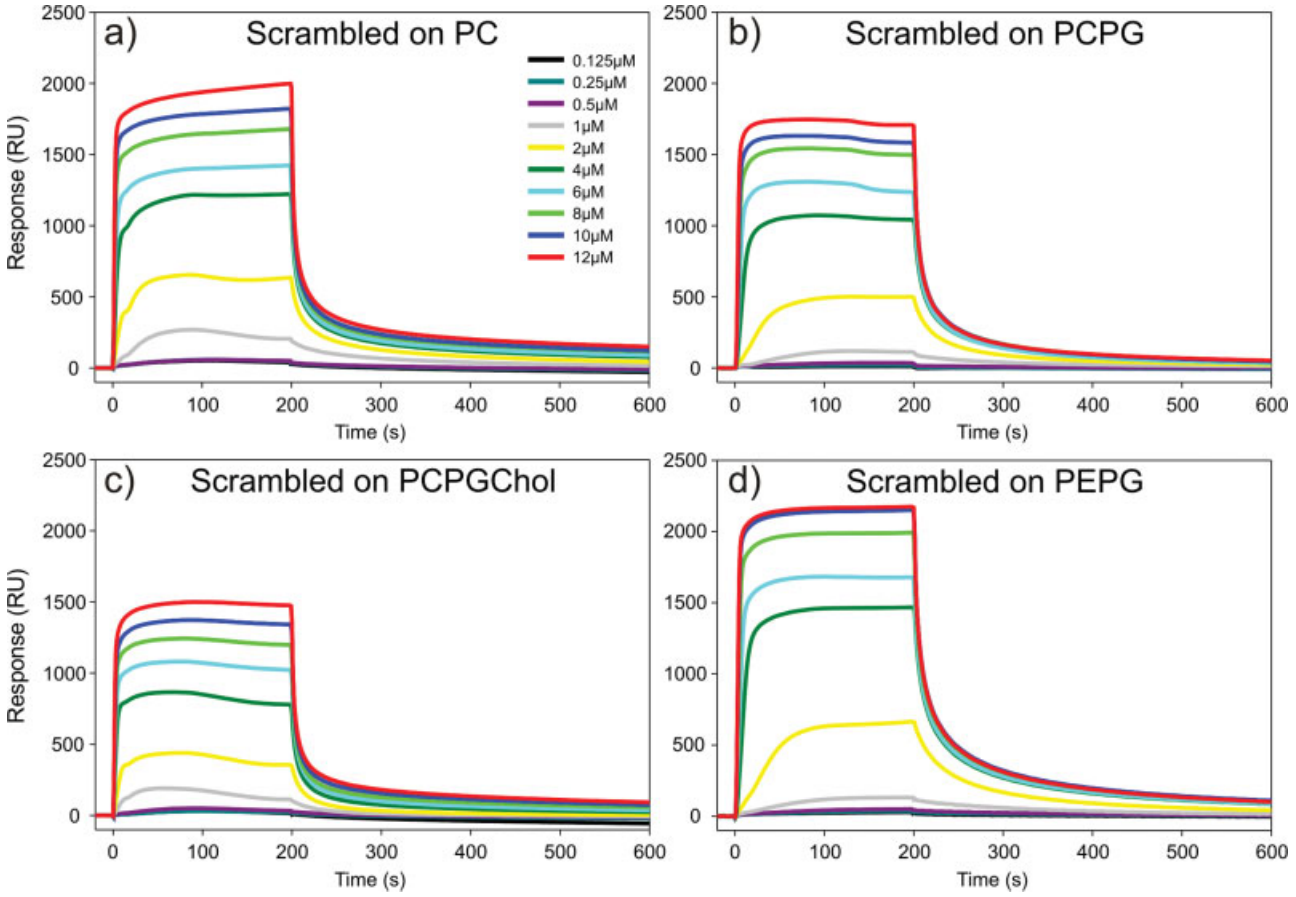


FIGURE 4 SPR sensorgrams of scrambled β-17 on the different lipid layer mixtures: (a) DMPC, (b) DMPC/DMPG (4:1), (c) DMPC/DMPG/cholesterol (16:4:5), (d) DMPE/DMPG (4:1) at 10 different concentrations from 0.125 to 12 μM. Injections were 200 s ($t = 0$–200 s) at 30 μl/min and the peptide was then allowed to dissociate for a further 400 s ($t = 200$–600 s) as buffer continued to flow through the system.

of 1500 RU. On completion of the injection, the peptide dissociated from the membrane quickly at first and then the rate slowed gradually. Although there was no peptide remaining on the surface with concentrations 0.125–2 μM, there was ∼300–400 RU remaining on the surface at 600 s for the higher concentrations (4–12 μM).

The binding of β-17 on DMPC/DMPG (4:1) was virtually identical to that on DMPC. There was a similar association rate increase with increasing concentration and the same fast association to a response of 1500 RU (with 12 μM) before reaching equilibrium for the remainder of the injection. The large response gap between the low and high concentrations and the dissociation was also similar with the higher concentrations (4–12 μM) having comparable amounts on the surface at 600 s (350–450 RU).

The results on DMPC/DMPG/cholesterol (16:4:5) were slightly different to those on the other lipids. Each concentration (from 2 to 12 μM) showed a similar fast rate of binding until reaching a saturation point. The top concentration reached a slightly lower response level compared with DMPC and DMPC/DMPG (4:1), which was just under 1500 RU. There was no large gap seen between the maximum response of the lower and higher concentrations that was seen on the previous two lipid layers and on this lipid, β-17 showed a linear increase in response with concentration. The dissociation rate was faster with this lipid layer with most of the peptide removed from the surface in the first 20 s after completion of the injection ($t = 200$–220 s).

β-17 showed a greater binding response on DMPE/DMPG (4:1) compared with the PC-containing lipids, with the top concentration binding quickly to 2250 RU. There was once again a gap between the response of the lower and higher concentrations. The overall change in association rate with concentration and the dissociation rate was similar to

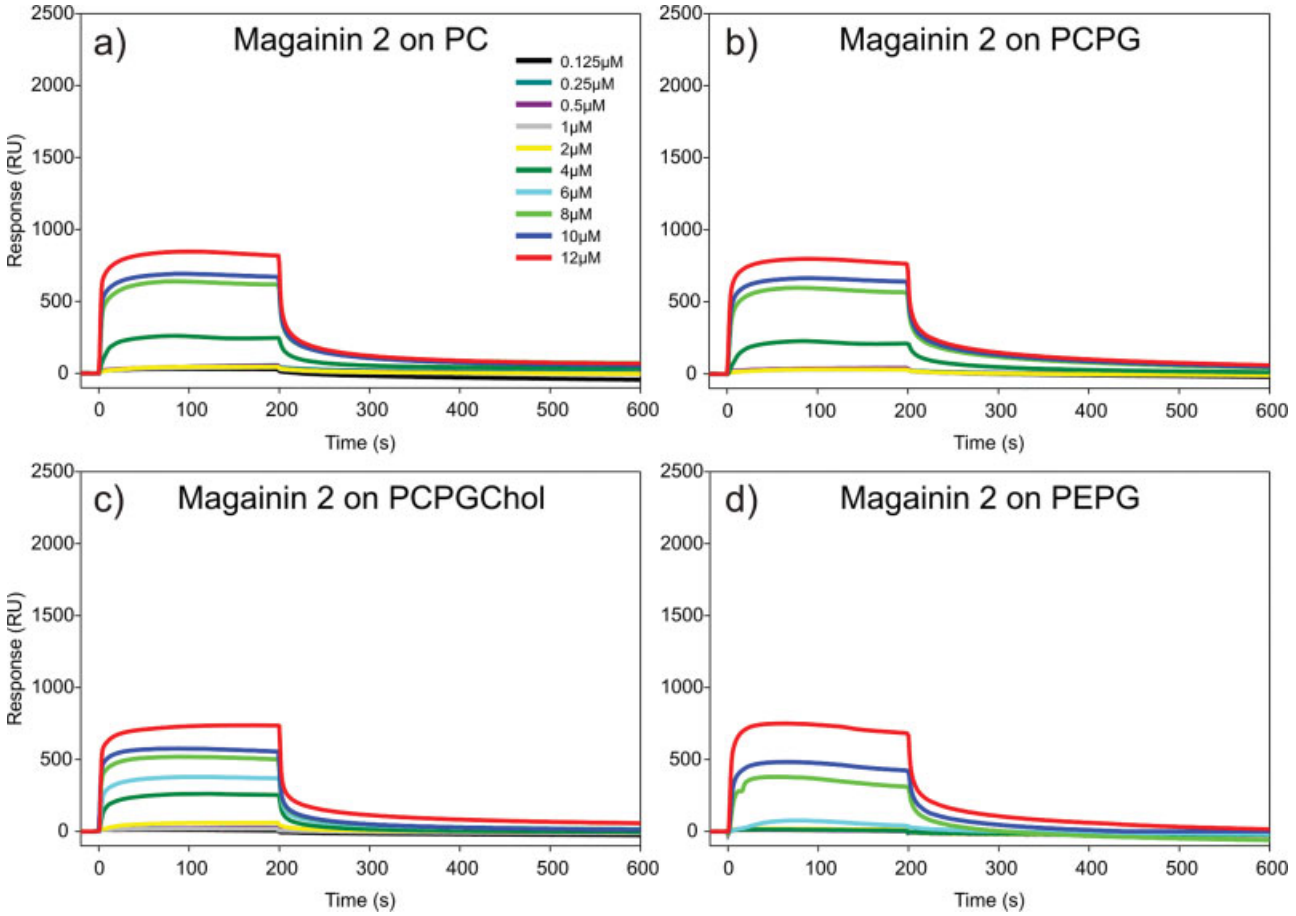


FIGURE 5 SPR sensorgrams of magainin 2 on the different lipid layer mixtures: (a) DMPC, (b) DMPC/DMPG (4:1), (c) DMPC/DMPG/cholesterol (16:4:5), (d) DMPE/DMPG (4:1) at 10 different concentrations from 0.125 to 12 μM. Injections were 200 s ($t = 0$–200 s) at 30 μl/min and the peptide was then allowed to dissociate for a further 400 s ($t = 200$–600 s) as buffer continued to flow through the system.

that of β-17 on DMPC and DMPC/DMPG (4:1). Again a similar amount of peptide remained on the surface after 600 s (~250–300 RU).

Scrambled β-17

Figure 4 shows typical sensorgrams for the binding of scrambled β-17 on the different lipid compositions and the results showed a concentration dependant increase in response on all lipids. The response of the scrambled peptide on DMPC was quite similar to that of β-17 on DMPC/DMPG/cholesterol (16:4:5), with a similar linear concentration dependant increase in response. The peptide showed a fast initial association that then approached equilibrium for the remainder of the injection. The level of binding was relatively higher with scrambled β-17 on DMPC with the top concentration (12 μM) reaching a maximum response around 2000 RU at the end of the injection. The dissociation rate was very fast with most of the peptide dissociated from the surface in the first few seconds after the end of injection.

The binding of scrambled β-17 on DMPC/DMPG (4:1) was similar to that on DMPC. The top concentration bound quickly again to a slightly lower maximum of ~1700 RU. The association, linear concentration dependant response and dissociation were all similar. The response on DMPC/DMPG/cholesterol (16:4:5) was similar again with the top concentration binding quickly to 1500 RU. Again the association, dissociation and curve saturation was comparable to scrambled β-17 on the other lipids. The peptide bound with the highest response on DMPE/DMPG (4:1) where the highest concentration reached 2200 RU. The association, dissociation, and curve separation was comparable in each case.

Magainin 2

Figure 5 shows typical sensorgrams for the binding of magainin 2 to the different lipid compositions. The binding to DMPC showed a linear concentration-dependent response, with a fast initial interaction that reached equilibrium for the remainder of the injection. Magainin 2 showed a relatively low response on DMPC with the top concentration reaching only ~750 RU compared with 1500–2000 RU for β-17 and scrambled β-17. There was a sharp decrease in response immediately following the end of injection with very little peptide remaining on the surface at 600 s. The response was similar on the DMPC/DMPG lipid layer, with a fast initial association that reached a similar response level to that on the DMPC layer. The peptide again reached equilibrium and showed a similar dissociation profile where most of the peptide dissociated from the surface at 600 s. Again the response of magainin 2 on DMPC/DMPG/cholesterol was similar although the peptide bound at slightly lower response levels to the previous two lipid systems. In addition, a greater separation between the concentration response levels was observed on this lipid layer. The response of magainin 2 on DMPE/DMPG was also similar to the DMPC-containing lipid mixtures and the dissociation was fast and the peptide completely dissociated from the membrane surface.

Quantitative Analysis of SPR Sensorgrams

Kinetic analysis of the sensorgrams shown in Figures 3–5 was performed via curve fitting to the langmuir, parallel and two state models as described in previous investigations.[16–19] As can be seen from the sensorgrams in Figures 3–5, the lower peptide concentrations bound poorly, but there was a transition between 2 and 6 μM to much higher levels of binding. These results demonstrate a change in the binding mechanism in this concentration range, which in turn prevents a quality fit to the data even with the two-state or parallel models. Even when only the four higher concentrations were used for kinetic analysis, poor fits were obtained. For example, for β17, the langmuir and parallel models consistently yielded χ^2-values between 200 and 3000 RU, and 70 and 2400 RU, respectively. The two-state model generally resulted in the best fit, with χ^2-values between 80 and 1000 RU for β17, and 25 and 220 RU for scrambled β17. The difficulty in using these results for comparative purposes is that the better fits were observed with different lipid mixtures, again preventing a useful comparison. Overall, much better fits were obtained for magainin 2, where the χ^2-values ranged from 20 to 250 RU for the langmuir model, 13–150 RU for the parallel model and 10–120 RU for the two-state model. However, the poor fits obtained for the two β-peptides prevented a meaningful comparison to be made using this data.

To allow a semiquantitative comparison between each peptide, the RU at the end of the association phase (at 200 s) was plotted against peptide concentration and shown in Figures 6a–6c for β-17, scrambed β-17, and magainin 2, respectively. These plots clearly illustrate the nonlinear dependence of RU on concentration between lower and higher concentrations and suggest that there is a threshold concentration below which minimal binding does not occur. Also plotted in Figure 6 is the RU at the end of the dissociation phase at 600 s. This data clearly illustrates the differences in the extent of dissociation between β-17 and scrambled β-17 whereby the scrambled peptide almost completely dissociates while there is ~20–30% residually bound β-17 depending on the bilayer composition. This phenomenon was further analyzed through the dependence on peptide concentration of the time from the beginning of the dissociation for the RU to reach 20% of the RU at 200 s, which is plotted in Figure 7. This data shows that β-17 dissociates slower than scrambled β-17 on DMPC, DMPC/DMPG, and DMPE/DMPG, but there is a rapid dissociation of both peptides from the cholesterol-containing mixture. A similar but less distinct trend was observed for magainin 2 with more residual bound peptide on DMPC and DMPC/DMPG than DMPC/DMPG/cholesterol and DMPE/DMPG.

DISCUSSION

Naturally derived antimicrobial peptides are highly diverse in terms of sequence, size, and structure often adopting amphipathic structures.[1] Based on previous investigations which have characterized the membrane interaction of magainin,[19] it was of considerable interest to compare the membrane binding properties of β-peptides with that of magainin 2. β-17 was designed as a β-peptide mimic of the naturally occurring magainin group of antimicrobial peptides.[2,14,24,25] β-17 comprises two different β-amino acid residues, namely APC and ACPC and has a three pentad repeat proceeded by two hydrophobic residues creating a 12-helix conformation (2.6 residues per turn and a pitch of 5.5 Å) of ~36 Å in length. The peptide is amphipathic with hydrophobic residues covering some 60% of one side of the helix and cationic residues 40% on the other side. The scrambled peptide contains the same amino acids but a different sequence in which the positive and hydrophobic residues are randomly distributed around the perimeter of the 12-helix resulting in the loss of amphipathicity (see Figure 1). This peptide therefore allowed the influence of amphipathicity on the lytic activity of β-17 to be evaluated.[26]

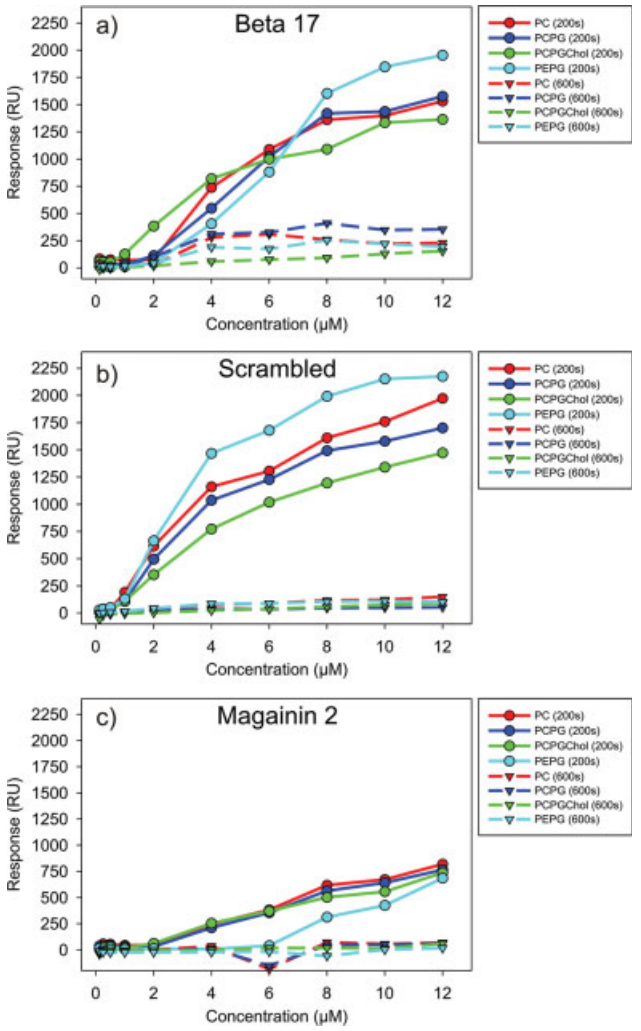


FIGURE 6 Plot of RU versus peptide concentration for (a) β-17, (b) scrambled β-17, and (c) magainin 2. Solid lines (and filled circles) correspond to RU at the end of the association phase at 200 s, and the dashed lines (and inverted triangles) correspond to RU at the end of the dissociation phase (600 s). Red = DMPC; blue = DMPC/DMPG (4:1); green = DMPC/DMPG/cholesterol (16:4:5), cyan = DMPE/DMPG (4:1).

It is generally accepted that the first step in the interaction of antimicrobial peptides with membranes is the initial approach to the membrane surface coupled with the induction of secondary structure.[1] The results of this study demonstrated that both β-peptides are highly structured, adopting a 12-helix[20] even in the absence of liposomes, so this step is unlikely to be a key differentiating component in the overall binding of these peptides. It is also significant that both β-peptides displayed partial 12-helix formation in buffer and water as this sets them apart from most α-peptides. Thus, secondary structure induction is likely to play a more significant role in the membrane interaction of magainin 2 which did not display α-helicity in buffer or DMPC as previously observed.[1,27] Interestingly, in contrast to β-peptides, a previous study[28] showed that scrambling the sequence of model amphipathic α-peptides had little effect on bioactivity or secondary structure and further illustrates that amphipathic secondary structure is an important determinant of cytolytic peptides.

The participation of electrostatic interactions in the initial binding coincides with the induction of secondary structure,[1] which is then followed by reorientation and/or insertion into the membrane mediated by hydrophobic interactions. β-17

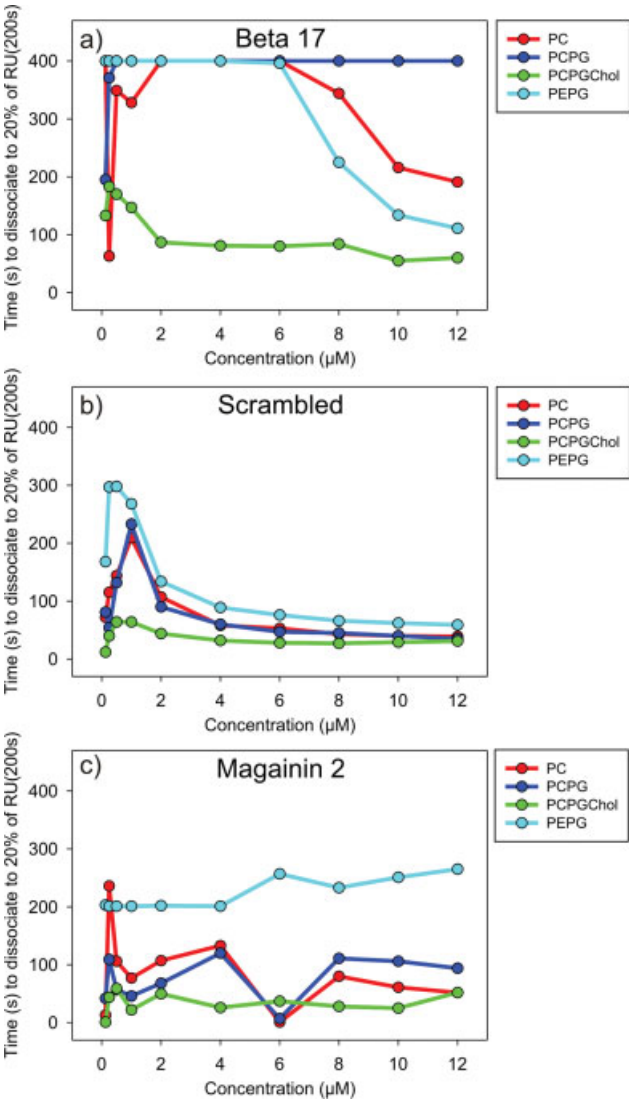


FIGURE 7 Plot of time (in seconds) to dissociate to 20% of RU (200 s) versus peptide concentration for (a) β-17, (b) scrambled β-17, and (c) magainin 2. Red = DMPC; blue = DMPC/DMPG (4:1); green = DMPC/DMPG/cholesterol (16:4:5), cyan = DMPE/DMPG (4:1).

and magainin 2 are both strongly amphipathic with the positively charged residues aligned on the face of the helix, while scrambled β-17 has the positive residues distributed around the helical surface. This large difference in the distribution of the positive residues did not result in any significant changes in the interaction of the two β-peptides with all four lipid mixtures. Moreover, while there were small but significant differences in the extent of binding on each lipid mixture (in the order of DMPE/DMPG > DMPC = DMPC/DMPG > DMPC/DMPG/cholesterol), this order was preserved for both β-peptides. The only marked difference was that scrambled β-17 almost completely dissociated from the membrane, while ~20–30% of β-17 remained bound at the end of the dissociation period. This result suggests that the amphipathic nature of β-17 allows it to penetrate the bilayer to a greater extent than scrambled β-17 which is non-amphipathic. Thus, the continuous stretch of hydrophobic residues on one face of β-17 assists the partial insertion of the peptide, whereas the non-continuous distribution of hydrophobicity on scrambled β-17 prevented this peptide from inserting into the membrane to the same extent as β-17.

Comparison of the membrane binding behavior of magainin 2 with both β-peptides reveals that the level of binding of magainin 2 on all four lipid mixtures was approximately half the level observed for each β-peptide. Moreover, the binding of magainin 2 was similar on all four lipid mixtures reflecting little selectivity between zwitterionic and anionic lipid mixtures. Although magainin 2 is strongly amphipathic, the overall hydrophobicity is lower given the relative hydrophobicity of the constituent α-amino acids compared with ACPC. These results suggest that the combination of the preformed amphipathic structure and the higher hydrophobicity of the hydrophobic face of the β-peptides facilitates a stronger interaction with all lipid mixtures than is possible for magainin 2.

The cytolytic properties of both β-peptides have been previously shown to be quite different, with β-17 exerting very potent activity, whereas scrambled β-17 is inactive (2). β-17 has also been shown to induce fluorescence leakage from liposomes and induce negative curvature of membranes by scanning microcalorimetry.[24] In contrast, most α-peptides including magainin 2 induce a positive curvature which suggests a different mechanism of interaction and insertion for β-peptides.[24,29] Moreover, β-17 was shown to have a stronger affinity for mixtures containing anionic phospholipids which was not observed in this study. However, these previous studies were performed with dioleylphospholipids with a much higher proportion of anionic phospholipids.[24] It is therefore possible that β-17 lyses the membranes by the formation of a pore-like structure, the structure of which is different from that induced by α-peptides as reflected in the difference in induced membrane curvature.

In summary, the results of the SPR experiments clearly show that the difference in biological potency between the two β-peptides is not related to any significant difference in initial binding behavior or the relative amount of peptide bound to the membrane, but rather differences in the degree of insertion and rate of membrane dissociation. The amphipathic structure of β-17 prevented complete dissociation suggesting a significant degree of membrane insertion, which was not achievable by the scrambled peptide. The most important structural difference relative to magainin 2 is their ability to form an amphipathic structure in any solution which may allow a different mechanism of binding and pore formation for β-17. Overall, the comparison of the membrane binding behavior of two helical β-peptides with an analogous α-peptide provides new insight into the combined role of secondary structure induction and amphipathicity in membrane binding and lysis of antimicrobial peptides.

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