**Functional analysis of an α-helical antimicrobial peptide derived from a novel mouse defensin-like gene**

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**Abstract**

Gene-encoded antimicrobial peptides (AMPs) are an essential component of the innate immune system in many species. Analysis of β-defensin gene expression in mouse tissue using primers that were specific for conserved sequences located outside of the β-defensin translated region identified a novel small gene. The novel gene had an open reading frame of 114 basepairs and encoded a predicted protein of 37 amino acid residues. A search of the genome database revealed that the gene locus and the sequence of exon 1 of this novel gene were similar to subgroup 1 mouse β-defensins. A small peptide, K17 (FSPQMLQDIIEKKTKIL), derived from the amino acid sequence of this novel gene was synthesized. Circular dichroism (CD) spectroscopic analysis of chemically synthesized peptide demonstrated that the peptide exhibited random coil conformation in aqueous solution, but the peptide adopted helical conformation in the presence of trifluoroethanol or sodium dodecyl sulphate, a membrane mimicking environment. The peptide exhibited bactericidal activity against *Salmonella enterica* serovar Typhimurium (Gram negative) and *Staphylococcus aureus* (Gram positive); it was not cytotoxic in cultures of mammalian cells or hemolytic in cultures of erythrocytes. These results suggested that K17 may be a candidate therapeutic for the treatment of bacterial infection.

Keywords: Antimicrobial peptide; Cationic α-helical peptide; defensin; bactericidal activity; CD spectroscopy

## Introduction

Innate immunity in animals depends in large part on the activity of nonspecific effector molecules. In particular, gene-encoded antimicrobial peptides (AMPs) are now clearly established as key players in both plant and animal defense systems. Despite broad divergence in sequence and taxonomy, most AMPs share a common mechanism of action involving membrane permeabilization of the pathogen cell membrane. Importantly, AMPs are recognized as a potential source of therapeutic drugs for the treatment of antibiotic-resistant bacterial infections.

To date, more than 880 different AMPs have been identified or predicted based on nucleic acid sequences. These include AMPs that are produced in many tissues and cell types in a variety of invertebrate, plant and animal species, as well as certain cytokines and chemokines. AMPs are a unique and diverse group of molecules that can be divided into subgroups based on amino acid composition and structure [1, 2]. The subgroup of anionic AMPs includes small peptides present in surfactant extracts, bronchoalveolar lavage fluid and airway epithelial cells. A second subgroup is comprised of approximately 290 linear cationic α-helical peptides. These peptides are short, lack cysteine residues and may possess a hinge or kink in the middle of the peptide. In aqueous solution, many of these peptides are disordered, but in the presence of trifluoroethanol (TFE) or sodium dodecyl sulphate (SDS) micelles, all or part of the molecules are converted to an α-helical conformation [1, 2]. For some of the members of this subgroup, α-helicity correlates with antimicrobial activity. A third subgroup contains approximately 44 cationic peptides that are rich in certain amino acids. This group includes the bactenecins and PR-39, which are rich in proline and arginine residues. Peptides in this group lack cysteine residues and are linear, although some can form extended coils. A forth subgroup of approximately 380 members consists of anionic and cationic peptides that contain cysteine residues and form disulfide bonds and stable β-sheets. This subgroup includes the diverse family of defensins and protegrin. There are approximately 55 α-defensins, 90 β-defensins, 54 arthropod (insect) defensins, and 58 plant defensins; a rhesus θ-defensin has also been identified.

Using a set of primers designed to amplify conserved mouse β-defensin sequences located outside of the translated region, we identified a novel small gene with an open reading frame of 114 basepairs (bp) and a predicted amino acid sequence of 37 residues.

A search of the genome database (http://www.ensembl.org) revealed that the gene locus and exon 1 sequence of this novel gene were similar to subgroup 1 mouse β-defensins. We designated this gene mouse β-defensin-like small (m*BDLs*).

The β-defensin genes consist of two exons. Exon 1 encodes a signal sequence and exon 2 encodes the pro and mature peptides that function as AMPs. The gene structure of m*BDLs* was similar to other β-defensins, which suggested that the mature peptide was encoded by exon 2. We synthesized a small peptide, K17, based on the nucleotide sequence of exon 2. K17 lacked cysteine residues. The peptide showed random coil conformation in aqueous solution, but formed α-helical structure in the presence of 50% TFE and 1% SDS, a membrane-mimicking environment, as identified by circular dichroism (CD) spectroscopy. The antimicrobial activity of K17 was assessed to determine if it functioned as an α-helical AMP. K17 exhibited bactericidal activity against Gram negative *Salmonella enterica* serovar Typhimurium and Gram positive *Staphylococcus aureus* at a concentration that is typical for other defensins. K17 did not exhibit cytotoxicity against normal or transformed mammalian cells (NIH3T3, HeLa, and A549 cells) or hemolytic activity against mouse erythrocytes at concentrations that inhibited bacterial growth. These results suggested that K17 may be a candidate therapeutic for the treatment of bacterial infections.

## Materials and Methods

### Tissue samples

Murine tissues were obtained from adult C57BL/6 mice. All animal experiments were approved by the Animal Care and Use Committee of the National Institute of Infectious Diseases.

### Reverse transcriptase (RT)-PCR

Total RNA was isolated from mouse brain tissue using Trizol (Invitrogen, Carlsbad, CA). Complementary DNA (cDNA) was generated by Omniscript RT PCR (Qiagen, Valencia, CA) and used as a template for RT-PCR. The PCR conditions were as follows: 95 °C for 2 minutes (min), followed by 30 cycles of 95 °C for 30 seconds (s), 55 °C for 30 s and 72 °C for 1 min, then 72 °C for 7 min. The primers (5'-CAGTCATGAGGATCCATTAC-3' and 5'-CATGGAGGAGCAAATTCTGG-3') were designed to target a conserved region of the mouse β-defensin gene sequence. PCR products were separated by agarose gel electrophoresis, stained with ethidium bromide and then visualized under UV illumination.

### 5'-Rapid amplification of cDNA ends (RACE) and sequencing

To isolate the full-length cDNA for m*BDLs*, we performed 5'-RACE using the 5'-Full RACE core kit (Takara, Tokyo, Japan) and the following primers: S1, 5'-TAGCCCTCAAATGCTGCAAG-3'; A1, 5'- AAGCTGCAAATGGAGACAGC-3; S2, 5'- AGACAAAGATCCTGTGAACC-3'; and A2, 5'-GCAACACCAGGAGAAATGAG-3'. PCR products were separated on a 2.0% agarose gel and the band of the expected size was removed, purified with a Qiaquick Gel Extraction Kit (Qiagen) and then subcloned into pGEM-T easy (Promega, Madison, WI). The cloned DNA sequence was confirmed by cycle sequencing using the Big Dye Terminator v1.1/3.1Cycle Sequencing Kit (Applied Biosystems, Foster City, CA) and a 3100-Avant Genetic Analyzer (Applied Biosystems).

### Genome, sequence, phylogenetic and secondary structure analyses

Genome analysis of the cloned cDNA and its locus was carried out using BLAST

searches of the Ensembl genome database (http://www.ensembl.org). DNASIS pro software (Hitachi Software Engineering, Tokyo, Japan) was used to predict amino acid sequences and perform sequence comparisons. Genetyx software (Genetyx Corporation, Tokyo, Japan) was used to construct the phylogenetic tree of *mBDLs* and five members of subgroup 1 mouse β-defensins. The PEP-FOLD Server (http://bioserv.rpbs.univ-paris-diderot.fr/PEP-FOLD) and Antimicrobial Peptide Database (APD; http://aps.unmc.edu/AP/main.html) were used to predict three dimensional (3D) conformations and predict antimicrobial function, respectively.

**Peptide synthesis**

Based on the predicted amino acid sequence of m*BDLs*, the small peptide K17 (FSPQMLQDIIEKKTKIL) and the K17 analogue A17, which contained three lysine-to-alanine substitutions (FSPQMLQDIIEAATAIL) were chemically synthesized by Sigma Genosys (Ishikari, Japan).

**Bactericidal assay**

Bactericidal assays were performed using wild-type and defensin-sensitive *phoP* strains of *Salmonella enterica* serovar Typhimurium *(S. typhimurium)* and *Staphylococcus aureus (S. aureus)*, as described previously [4]. Bacteria were stored at -80 °C in cryopresevant. All assays were performed in duplicate. Bacteria were cultured in Tryptic Soy Broth (TSB). One thousand colony forming units (CFU), as determined by the OD of exponentially growing cultures, were collected by centrifugation and then resuspended in 25 µl of distilled water. Various concentrations of peptide were added to the cells, and the suspension was allowed to incubate for 60 min at 37 °C, at which point CFU were quantified by growth on Tryptic Soy Agar (TSA) plates at 37 °C overnight. The number of CFU of treated samples was compared to controls without peptide.

**Growth inhibitory activity against tumor and normal fibroblast cells**

Cells were grown in DMEM supplemented with 10% heat-inactivated fetal bovine serum (FBS), 100 units/ml penicillin G and 100 µg/ml streptomycin. Cells were plated on 96-well plates at a concentration of 6 x $10^3$ cells/100 µl in the same medium. After

incubating the plates overnight at 37 °C in an atmosphere of 5% $CO_2$, the medium was changed to OPTI-Pro without FBS. Ten µl of diluted peptide were added to the cells, and then the plates were incubated for 24 hours (hr), at which point 50 µl of XTT solution from the Cell proliferation kit II [XTT] (Roche Diagnostics, Indianapolis, IN) were added to each well. The plates were incubated for an additional 3 hr, and then absorbance was measured at 490 nm using a microplate ELISA reader (Bio-Rad Laboratories, Hercules, CA).

**Hemolysis assay**

Hemolytic activity was evaluated by measuring hemoglobin release from fresh mouse erythrocytes. Mouse red blood cells were isolated using a cellulose column. Erythrocytes were suspended in phosphate buffered saline (PBS; 100 µl) (8% v/v) and then plated on 96-well U-bottomed plates (Nunc, Roskilde, Denmark). Ten µl of peptide dissolved in PBS were added to each well, and then the plates were incubated for 1 hr at 37 °C, after which they were subjected to centrifugation at 1,500 x *g* for 5 min. The supernatant (100 µl) was then transferred to a 96-well microtiter plate. Hemolysis was monitored by absorbance at 414 nm with an ELISA plate reader (Bio-Rad). Complete hemolysis (set as 100%) was achieved by the addition of Triton X-100 at a final concentration of 0.01%. Hemolysis percentage was calculated according to the following equation: % hemolysis = [($Ab_{414}$ nm in peptide solution-$Ab_{414}$ nm in PBS)/($Ab_{414}$ nm in 0.1% Triton X-100-$Ab_{414}$ nm in PBS)] x 100.

**CD spectroscopy**

The far-UV CD spectra of K17 and A17 were recorded in a J-805 spectropolarimeter (Jasco, Tokyo, Japan) at room temperature using 0.2 cm path length quartz cuvette. Both peptides were dissolved in either distilled water, 1% SDS, or 50% TFE at final concentrations of 50 and 100 µg/ml for K17 and A17, respectively.

**Statistics**

Experimental groups were compared statistically using the Student's *t*-test. A *P* value of $<0.05$ was considered statistically significant.

## Results

### Identification of the m*BDLs* gene

RT-PCR analysis of a mouse brain cDNA library was carried out using primers that were specific for conserved mouse β-defensin sequences located outside of the translated region. Amplified products were separated by agarose gel electrophoresis and a small band of approximately 150 bp, which was similar to the expected size of mouse β-defensin genes, was detected (data not shown). To characterize this small PCR product, the amplified fragment was subcloned and sequenced. Analysis by 5'-RACE using a primer designed to target the coding region revealed that the small cDNA contained a 114 bp open reading frame encoding a putative peptide of 37 amino acid residues (Fig. 1A).

### *In silico* genome scan

The small cDNA sequence was verified using the Emsembl BLASTview program [5]. There was a 100% match between the isolated small cDNA and a locus on mouse chromosome 8 (database location: AC121131.14, 100856 to 100918). The gene contained 2 exons separated by 1 intron and was 1698 bp in length (Fig. 1B). In exon 1, the 5'-untranslated region contained an NF-κB binding site and TATA box (Fig. 1B). The gene structure was consistent with characteristics commonly found in mouse defensin genes, namely, 2 exons separated by an intron (~1.7 kb) and an NF-κB binding site and TATA box in the 5'- untranslated region [6].

The A3 region on chromosome 8 is divided into two contigs that are separated by approximately 850 kb. The first contig contains six mouse β-defensin (mBD) genes, including mBD4, 6, 5, 3 and 7, as well as DefR1. These genes all belong to β-defensin subgroup 1. The small gene was located after mBD7 in the first contig (Fig. 1C). Since the small gene had similar structural characteristics to other mouse β-defensins, it was designated m*BDLs*, for mouse β-defensin-like small gene.

### Prediction of mBDLs amino acid sequence

The amino acid sequence of mBDLs was predicted and compared with other members of the mouse β-defensin family (Fig. 2A). The cDNA sequence of m*BDLs* exhibited 60-80% similarity to other subgroup 1 members such as mBD3, mBD4, mBD5, mBD7 and

DefR1, but lower homology to subgroup 2 members, including mBD2 and mBD10. The prepro region (amino acids 1-23) of the mBDLs peptide was highly similar to subgroup 1 family members, but the level of similarity was lower for the mature peptide sequence (amino acids 24-66; Fig. 2A). It has been reported that β-defensin subgroup 1 members contain similar signal sequences and are of similar exon length [7]. As both the sequence and length of m*BDLs* exon 1 were similar to other subgroup 1 members, we propose that it belongs to the same family. Phylogenetic analysis of mBDLs and five mouse subgroup 1 β-defensin genes showed that mBDLs forms a branch with mBD7, while mBD4 and mBD5 with mBD3 formed other branches (Fig. 2B). Although mBDLs exhibited similarity to other β-defensin subgroup 1 family members with respect to gene locus and exon-intron structure, as well as by phylogenetic analysis, mBDLs was much smaller than these other defensins and did not contain cysteine residues.

**Design of peptides and CD analysis of these peptides**

The mature defensin peptide is encoded by exon 2 [8]. Based on the predicted amino acid sequence of m*BDLs* exon 2, we synthesized a small peptide, termed K17 (FSPQMLQDIIEKKTKIL), and the K17 analogue A17 (FSPQMLQDIIEAATAIL), which contained three alanines in place of the three lysine residues (Fig. 3A). Structural analysis using PEP-FOLD, which builds on a new *de novo* approach to predicting 3D peptide structures from sequence information [3], indicated that K17 and A17 each adopt an α-helical conformation (Figs. 3B and C). The secondary structures of these peptides were analyzed by a CD spectroscopy. The CD spectra of K17 and A17 dissolved in distilled water exhibited disordered as random coil (Figs. 3D and E). Upon the addition of 50% TFE or 1% SDS, a conformational change occurred, as evidenced by spectrum containing minima at 208 and 222 nm. Based on these spectra, these peptides adopted α-helical structure in the membrane-mimicking environment. The result is in good agreement with the results of other online prediction, Antimicrobial Peptide Database (APD) that predicts whether these peptides would have antimicrobial activity [10]. According to the APD, K17, which was cationic and could putatively interact with membranes, was predicted to function as an AMP, while A17, which had a net negative charge, would not.

**Antimicrobial activity**

The bactericidal activity of the two peptides was first examined using the defensin-sensitive *phoP* strain of *S. typhimurium*. The lack of a *PhoP* system for antimicrobial resistance makes this strain a useful tool for determining the antimicrobial mechanisms of defensins [4]. At concentrations (5 and 10 μg/ml) that are generally considered optimal for the bactericidal activity of other AMPs, K17 exhibited dose-dependent bactericidal activity against the *phoP*-strain of *S. typhimurium*, whereas there was no increase in activity in the presence of A17 (5 and 10 μg/ml) (Fig. 4A). We next examined bactericidal activity against wild-type *S. typhimurium* (Gram negative) and *S. aureus* (Gram positive). K17 showed significantly higher bactericidal activity against *S. typhimurium* and *S. aureus* (approximately 40%) as compared to A17 (Fig. 4B).

**Cytotoxicity against mammalian tumor and normal cells**

The cytotoxicity of K17 and A17 in mammalian cell cultures was investigated by growth inhibition of normal mouse NIH3T3 fibroblasts and two transformed tumor cell lines (HeLa and A549). Neither K17 nor A17 was cytotoxic in cultures of normal and tumor cells at concentrations of 2, 20, and 200 μg/ml (Fig. 4C).

**Hemolytic activity**

The hemolytic activity of K17 and A17 was determined using mouse erythrocytes. No significant hemolytic activity of the peptides was observed at concentrations of 2, 20, and 200 μg/ml (Fig. 4D).

**Discussion**

A mouse β-defensin-like gene was amplified from mouse brain using primers designed to target conserved mouse β-defensin gene sequences. This novel gene was designated mouse β-defensin-like small, or m*BDLs*. The genomic structure of m*BDLs* was similar to other subgroup 1 β-defensins in that it contained 2 exons separated by 1 intron, was approximately 2 kb [6,11,12], and contained an NF-κB binding site [6,13,14] and TATA box [11] in the 5'-untranslated region.

The β-defensin genes possess two exons and one intron. A signal sequence is encoded by exon 1 and the mature peptide is encoded by exon 2 [8]. Because the sequence of exon 1 of m*BDLs* was similar to other subgroup 1 members, we predicted that exon 2 would encode the mature peptide. We synthesized a small peptide, termed K17, derived from the nucleotide sequence of exon 2 of m*BDLs*. We also synthesized a K17 analogue that contained alanine residues in place of lysine residues, termed A17. Replacement or deletion of specific amino acid residues in the original peptide is a common technique for analyzing new AMPs [15], such as cecroprins and maganines [16, 17]. K17 was cationic, lacked cysteine residues, and it was shown that the peptide exhibited α-helical conformation in lipid membrane-mimicking solvents such as 50% TFE or 1% SDS by CD spectroscopy. A17 was also adopted α-helical structure but had negative charge.

AMPs are divided into four subgroups based on amino acid composition and structure. One of AMPs subgroups is typically α-helical, short, cationic peptides that lack cysteine residues [1]. The characteristics of K17 were consistent with those of the AMP subgroup. Furthermore, the APD predicted that K17 would function as an AMP [10]. Examination of the antimicrobial activity of K17 and A17 against the *phoP*-strain of *S. typhimurium*, which is sensitive to AMPs [4], and two wild-type strains (*S. typhimurium* and *S. aureus*) showed that K17 had antimicrobial activity against Gram positive and Gram negative bacteria at concentrations that are generally considered optimal for the bactericidal activity of other AMPs. The K17 analogue A17, which also adopted an α-helical conformation but lost charge due to replacement of lysines by alanines, lacked antimicrobial activity. While the precise mechanism of action of AMPs is not fully understood [18], these results suggested that charge is related to antimicrobial activity, at least for K17. Although some α-helical antimicrobial peptides exert cytotoxic and hemolytic effects [19], K17 was not hemolytic in cultures of mouse erythrocytes or

cytotoxic in cultures of NIH3T3, HeLa or A549 cells. These results suggest that K17 is non-toxic to mammalian cells at concentrations that are bactericidal.

In summary, we have isolated a novel β-defensin-like gene, m*BDLs*, and have demonstrated that a peptide based on the m*BDLs* sequence possesses antimicrobial activity against both Gram negative and Gram positive bacteria. Although the peptide does not contain any cysteine residues, as do other defensins, it possesses many features that are characteristic of one of AMPs subgroups. As such, K17 represents a potential candidate therapeutic for the treatment of bacterial infections.

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**Figure Legends**

Fig. 1 Structure of a small gene with homology to mouse β-defensins
(A) Nucleotide sequence of the small cDNA amplified using primers specific for conserved mouse β-defensin sequences, and the corresponding predicted amino acid sequence. * indicates the stop codon. (B) The full genomic sequence of the small gene. The gene contains 2 exons separated by 1 intron and is 1698 bp in length. A NF-κB binding site is underlined and a TATA box is double-underlined. Coding sequences are represented in bold and italic. (C) The small gene and other mouse β-defensins localize to the A3 region of chromosome 8 (Chr 8). The small gene is located after mBD7 in the first contig. 4, mBD4; 6, mBD6; 5, mBD5; 3, mBD3; R1, DefR1; 7, mBD7; Ls, mBDLs. .

Fig. 2 Comparison of the amino acid sequences of mBDLs and other mouse β-defensins
(A) Alignment of amino acid sequences of mBDLs and other mouse β-defensins of subgroups 1 and 2. Amino acid residues that are conserved in 80% of subgroup members are shaded in gray. (B) Phylogenetic tree of mBDLs and 5 mouse β-defensins of subgroup 1.

Fig. 3 Secondary structures of the peptide derived from mBDLs sequence
(A) Amino acid sequences of K17 and the K17 analog, A17. Peptide sequences were based on the m*BDLs* exon 2 nucleotide sequence. (B and C) Estimated three-dimensional structures of K17 and A17 generated using PEP-FOLD software. The lowest energy model among the predicted structures is shown. Black color in K17 corresponds to main chain lysine residues (arrow heads). Estimated structure of A17, in which Lys-12, 13, and 15 of K17 were replaced with alanine. Gray color in A17 corresponds to main chain alanine residues. Representations were generated using Accelrys Discovery Studio software. (D and E) CD spectra of K17 (D) and A17 (E) under different conditions. The far-UV CD spectra of K17 (50 μg/ml) and A17 (100 μg/ml) was recorded in distilled water (solid line), 1% SDS (dotted line), or 50% TFE (dashed line).

Fig. 4 Functional analysis of K17

(A) Bactericidal activity of K17 and A17 against the defensin-sensitive *phoP*-strain of *S. typhimurium*. *, $p<0.05$. Mean CFUs were used to estimate survival. Data represents the percentage of bacteria killed in the peptide-treated sample as compared to the control (no peptide). (B) Bactericidal activity of K17 and A17 (5 μg/ml) against wild-type *S. typhimurium* and *S. aureus*. *, $p<0.05$. (C) Viability of NIH3T3, HeLa and A549 cells incubated with the indicated concentrations (2, 20, 200 μg/ml) of K17 (◆) and A17 (■). (D) Hemolytic activity of the indicated concentrations of K17 (◆) and A17 (■) in cultures of mouse erythrocytes.

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# Figure 1

## (A)

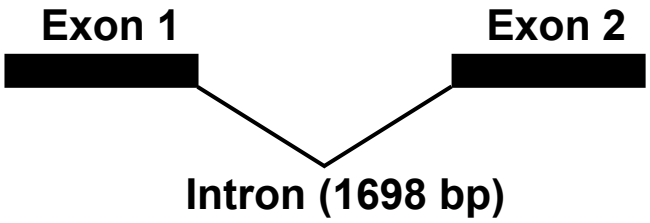
Sequence (114 bp)

ATGAGGATCCGTTACCTTCTG
TTCTCATTTCTCCTGGTGTTG
CTGTCTCCATTTGCAGCTTTT
AGCCCTCAAATGCTGCAAGAT
ATAATAGAGAAGAAGACAAAG
ATCCTGTGA

Amino acid sequence
(37 amino acids)

MRIRYLLFSFLLVLLSPFA
AFSPQMLQDIIEKKTKIL*

## (B)




AACCAGTAAGTCTCTCCCAGGACAGCCTGGG
TCCCTCTCATGTAAGATGTAAGGCAGGAAGC
TGTTCTTGTTGAGCAGTGCAGGAGGAAATCA
CCTGGGGATCCTCACATTTGCATAAGAGACT
CTGAGTGTGCTCTCCAATGTCACCTTGACAA
GAGAGATAAGGTGCTCTGTGTTTCATAATTG
TAATTCCTTGGATTCAAGTCAGTGTAGAATC
CTACCGAGGAAGCAGCACCTGGCACTATATA
AGGCACTGAGCTCAAGTCCCTCTGCATCTCT
GTACCTCACCAGGCTTCAGTC

***ATGAGGATCCGTTACCTTCTGTTCTCATTTC***
***TCCTGGTGTTGCTGTCTCCATTTGCAG***

1698 bp of intron sequence

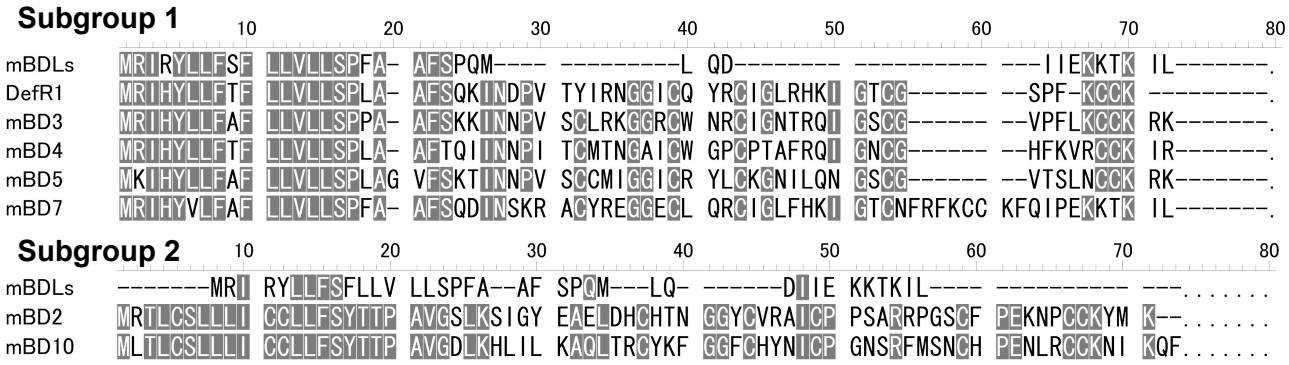
***CTTTTAGCCCTCAAATGCTGCAAGATATAA***
***TAGAGAAGAAGACAAAGATCCTGTGA***

NF-κB binding site
TATA box

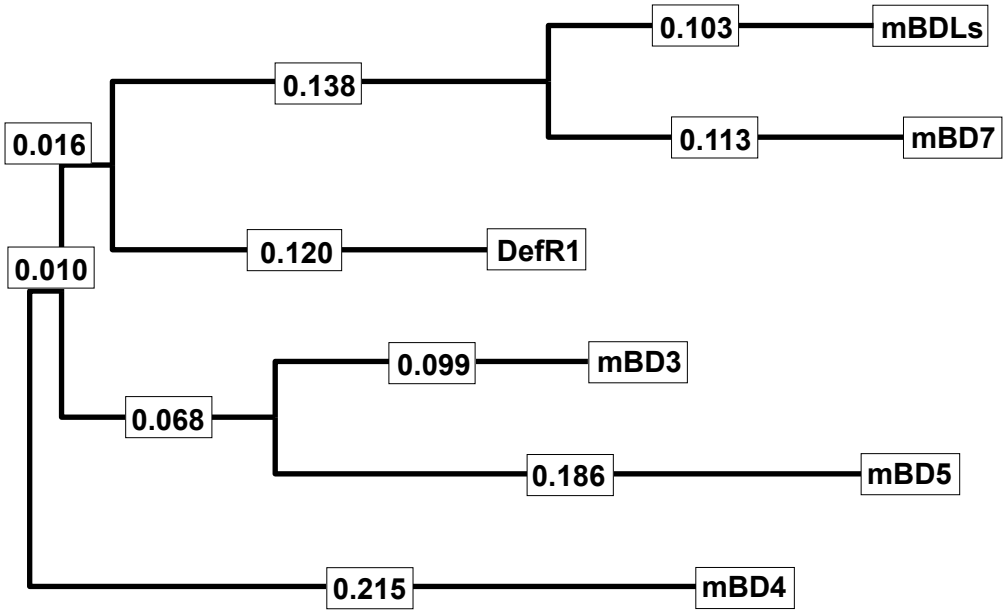
## (C)

# Figure 2

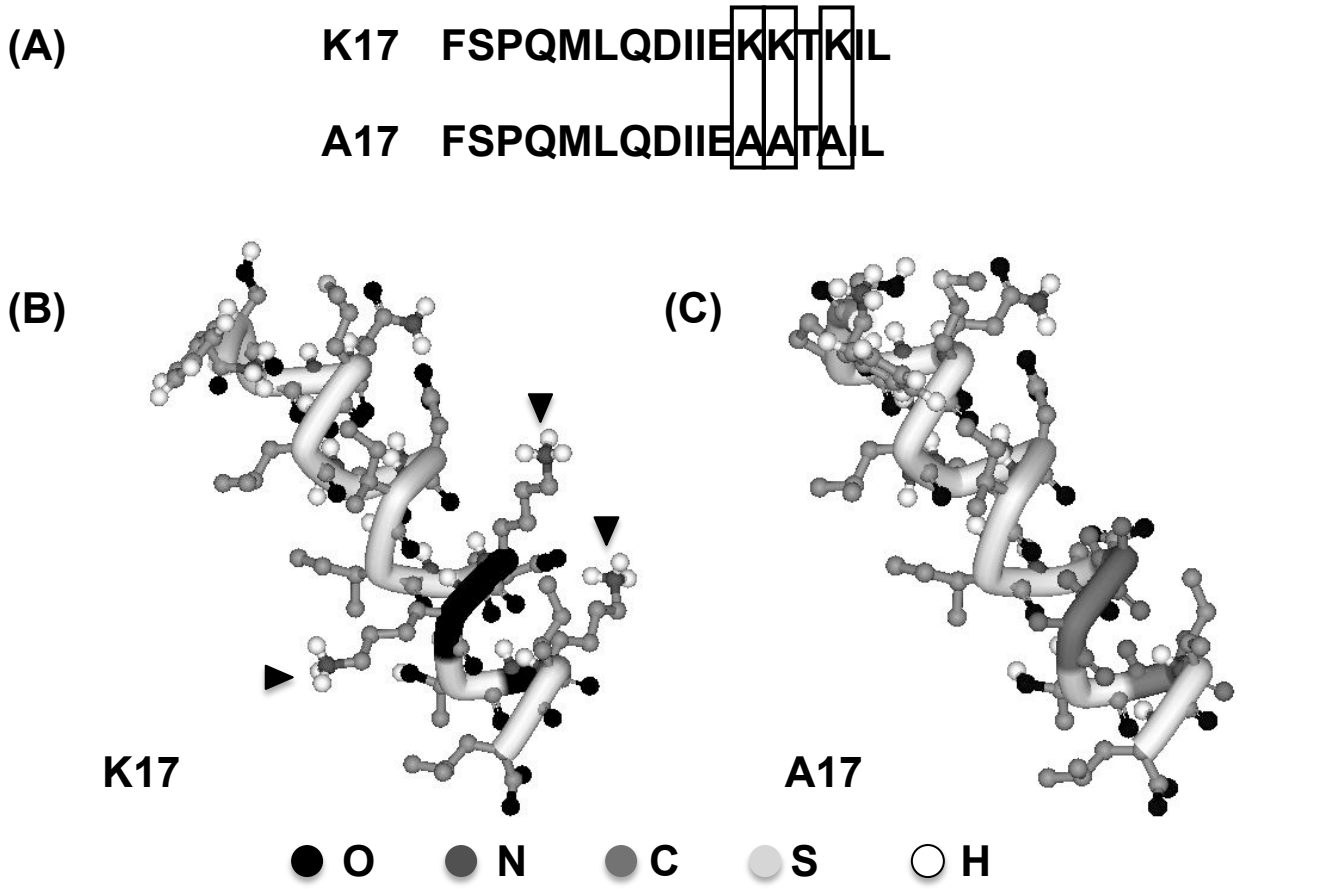
## (A)

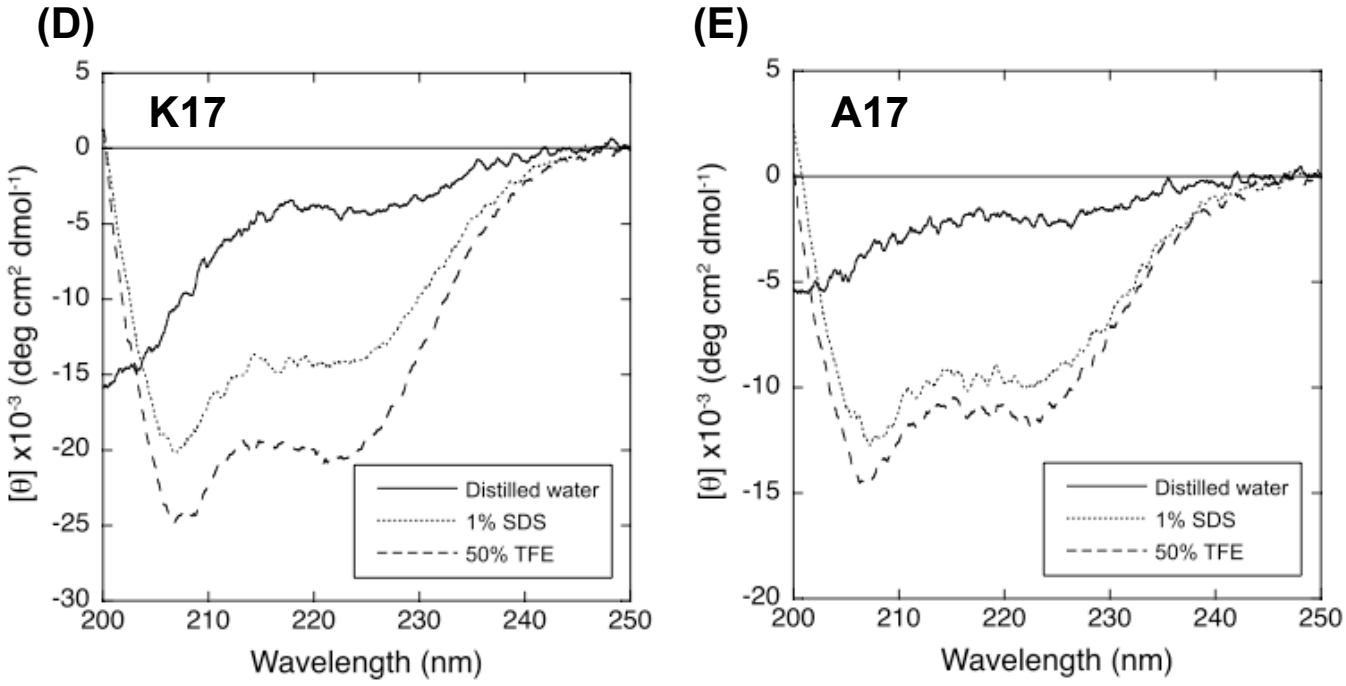

Subgroup 1
10 20 30 40 50 60 70 80
mBDLs MRIRYLLFSF LLVLLSPFA- AFSPQM---- ---------L QD-------- ---------- ---IIEKKTK IL-------.
DefR1 MRIHYLLFTF LLVLLSPLA- AFSQKINDPV TYIRNGGICQ YRCIGLRHKI GTCG------ --SPF-KCCK ---------.
mBD3 MRIHYLLFAF LLVLLSPPA- AFSKKINNPV SCLRKGGRCW NRCIGNTRQI GSCG------ --VPFLKCCK RK-------.
mBD4 MRIHYLLFTF LLVLLSPLA- AFTQIINNPI TCMTNGAICW GPCPTAFRQI GNCG------ --HFKVRCCK IR-------.
mBD5 MKIHYLLFAF LLVLLSPLAG VFSKTINNPV SCCMIGGICR YLCKGNILQN GSCG------ --VTSLNCCK RK-------.
mBD7 MRIHYVLFAF LLVLLSPFA- AFSQDINSKR ACYREGGECL QRCIGLFHKI GTCNFRFKCC KFQIPEKKTK IL-------.
Subgroup 2
10 20 30 40 50 60 70 80
mBDLs -------MRI RYLLFSFLLV LLSPFA--AF SPCM---LQ- ------DIIE KKTKIL---- ---------- ---.......
mBD2 MRTLCSLLLI CCLLFSYTTP AVGSLKSIGY EAELDHCHTN GGYCVRAICP PSARRPGSCF PEKNPCCKYM K--.......
mBD10 MLTLCSLLLI CCLLFSYTTP AVGDLKHLIL KAQLTRCYKF GGFCHYNICP GNSRFMSNCH PENLRCCKNI KQF.......

## (B)

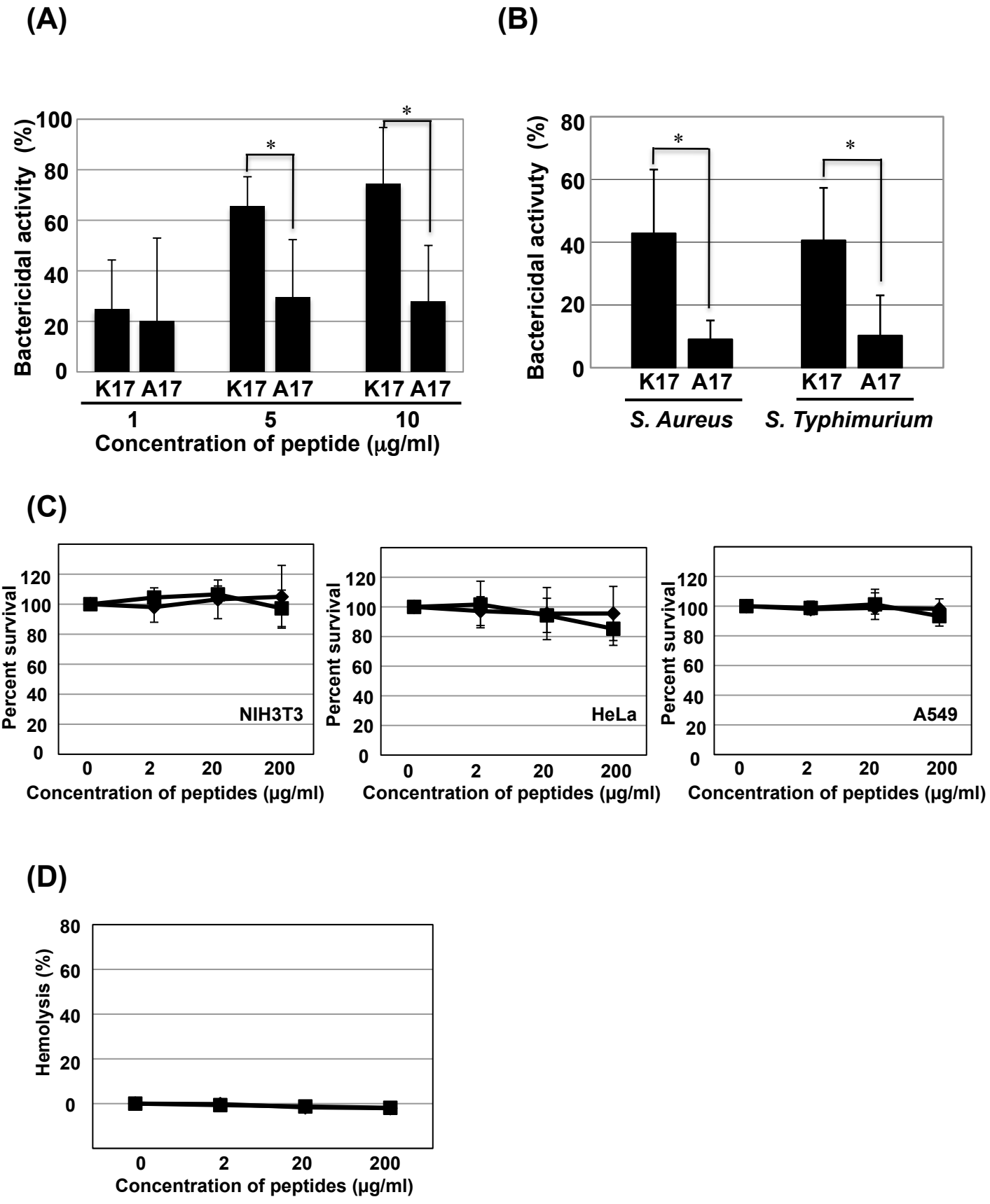

0.103
mBDLs
0.138
0.113
mBD7
0.016
0.120
DefR1
0.010
0.099
mBD3
0.068
0.186
mBD5
0.215
mBD4

# Figure 3


(A)
K17 FSPQMLQDIIEKKTKIL
A17 FSPQMLQDIIEAATAIL
(B)
(C)
K17
A17
O
N
C
S
H


(D)
K17
[θ] x10-3 (deg cm2 dmol-1)
Wavelength (nm)
Distilled water
1% SDS
50% TFE
(E)
A17
[θ] x10-3 (deg cm2 dmol-1)
Wavelength (nm)
Distilled water
1% SDS
50% TFE

# Figure 4


(A)
Bactericidal activity (%)
100
80
60
40
20
0
*
*
K17 A17
K17 A17
K17 A17
1
5
10
Concentration of peptide (μg/ml)
(B)
Bactericidal activuty (%)
80
60
40
20
0
*
*
K17 A17
K17 A17
S. Aureus
S. Typhimurium
(C)
Percent survival
120
100
80
60
40
20
0
NIH3T3
0 2 20 200
Concentration of peptides (μg/ml)
HeLa
A549
(D)
Hemolysis (%)
80
60
40
20
0
0 2 20 200
Concentration of peptides (μg/ml)