Supplementary data

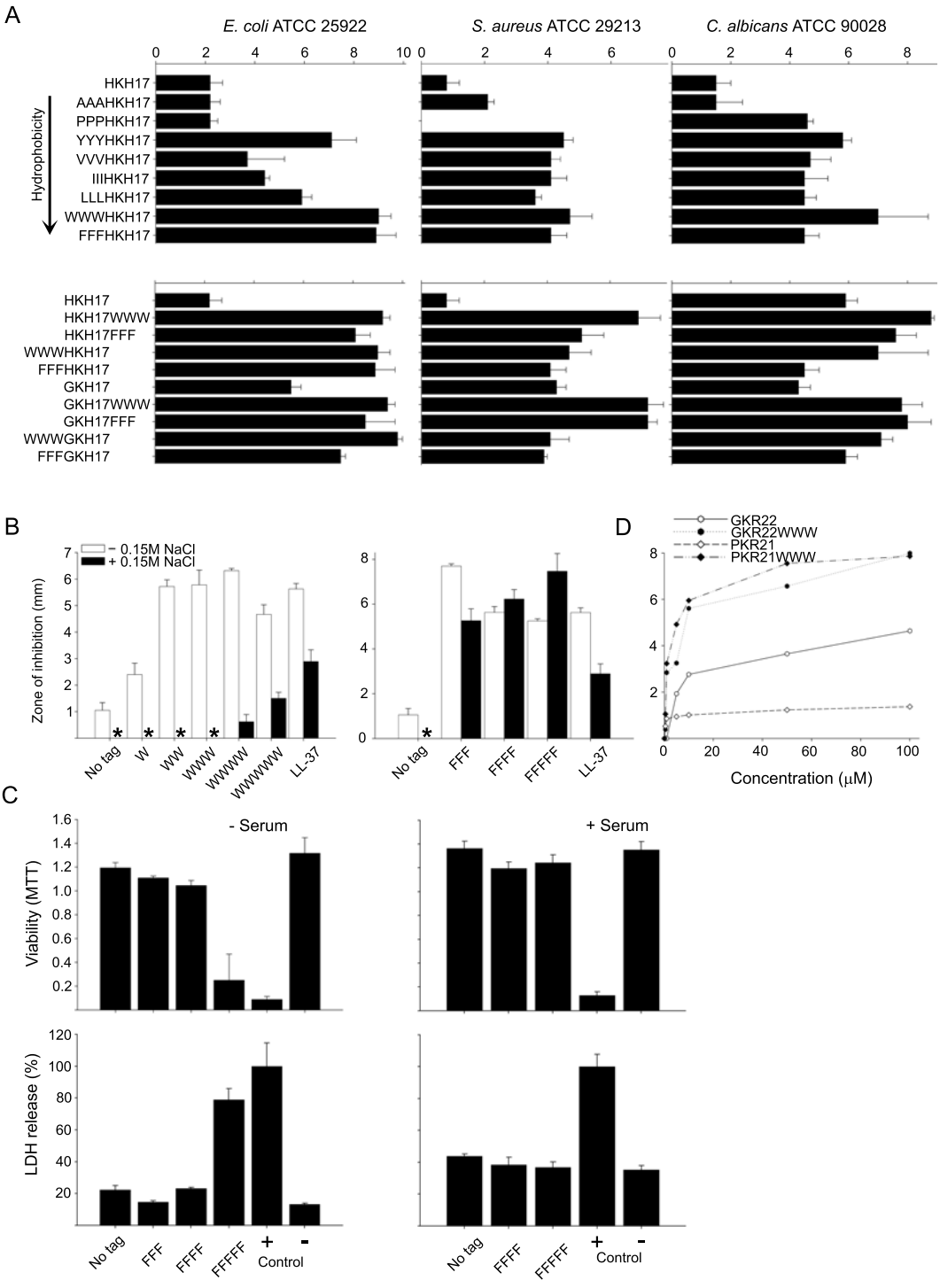
Table 1 Amino acid sequences, antimicrobial effects, and hydrophobicity of peptides analysed. For determination of antimicrobial activities, *S. aureus* ATCC 29213, *E. coli* ATCC 25922 ($4x10^6$ CFU), or *C. albicans* ATCC 90028 ($1x10^5$ CFU) isolates were inoculated in 0.1% TSB agarose gel. Each 4 mm-diameter well was loaded with 6 µl of peptide (at 100 µM). The zones of clearance correspond to the inhibitory effect of each peptide after incubation at 37 °C for 18-24 h (mean values are presented, n=3). The hydrophobicity of peptides according to CCS (combined consensus hydrophobicity scale)**,** Eisenberg and Kyte & Doolittle scales are provided.

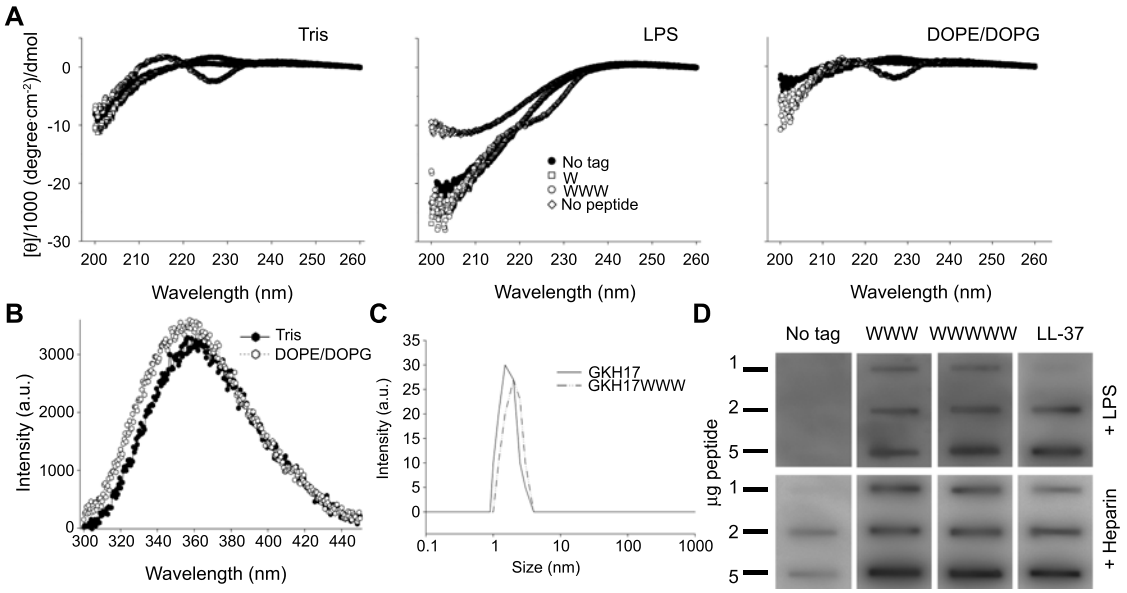
		RDA (zone of inhibition)			Hydrophobicity		
Modification	Sequence	*C. albicans* ATCC 90028	*E. coli* ATCC 25922	*S. aureus* ATCC 29213	CCS	Kyte and Doolittle	Eisenberg
Control	HKHGHGHGKHKNKGKKN	1.4	2.2	0.8	-6.0	-3.0	-0.8
C-terminal	HKHGHGHGKHKNKGKKN**L**	3.2	2.7	2.9	-5.2	-2.7	-0.7
	HKHGHGHGKHKNKGKKN**LL**	3.3	3.8	3.8	-4.4	-2.3	-0.7
	HKHGHGHGKHKNKGKKN**LLL**	4.7	4.9	5.7	-3.7	-2.0	-0.6
	HKHGHGHGKHKNKGKKN**WWW**	8.8	9.2	6.9	-3.7	-2.7	-0.6
	HKHGHGHGKHKNKGKKN**FFF**	7.6	8.1	5.1	-3.6	-2.2	-0.6
Control	GKHKNKGKKNGKHNGWK	3.8	5.9	3.2	-5.8	-3.0	-0.8
C-terminal	GKHKNKGKKNGKHNGWK**L**	4.4	6.1	4.4	-4.9	-2.6	-0.8
	GKHKNKGKKNGKHNGWK**LL**	5.5	10.2	4.3	-4.2	-2.3	-0.7
	GKHKNKGKKNGKHNGWK**LLL**	4.1	9.6	4.9	-3.5	-2.0	-0.6
	GKHKNKGKKNGKHNGWK**WWW**	7.8	9.4	7.2	-3.5	-2.7	-0.7
	GKHKNKGKKNGKHNGWK**FFF**	8.0	8.5	7.2	-3.4	-2.1	-0.6
N-terminal	**L**HKHGHGHGKHKNKGKKN	0.9	2.3	2.0	-5.2	-2.7	-0.7
	LLHKHGHGHGKHKNKGKKN	1.9	2.8	2.9	-4.4	-2.3	-0.7
	LLLHKHGHGHGKHKNKGKKN	4.5	5.9	3.6	-3.7	-2.0	-0.6
	AAAHKHGHGHGKHKNKGKKN	1.5	2.2	2.1	-5.3	-2.3	-0.6
	IIIHKHGHGHGKHKNKGKKN	4.5	4.4	4.1	-3.8	-1.9	-0.6
	VVVHKHGHGHGKHKNKGKKN	4.7	3.7	4.1	-4.5	-2.5	-0.6
	PPPHKHGHGHGKHKNKGKKN	4.6	2.2	0.0	-5.2	-3.1	-0.7
	YYYHKHGHGHGKHKNKGKKN	5.8	7.1	4.5	-4.8	-2.8	-0.7
	FHKHGHGHGKHKNKGKKN	6.1	7.9	4.4	-5.2	-2.7	-0.7
	FFHKHGHGHGKHKNKGKKN	3.7	2.3	3.3	-4.4	-2.4	-0.6
	FFFHKHGHGHGKHKNKGKKN	4.5	8.9	4.1	-3.6	-2.2	-0.6
	WHKHGHGHGKHKNKGKKN	4.0	4.3	4.1	-5.2	-2.9	-0.7
	WWHKHGHGHGKHKNKGKKN	5.1	3.5	3.6	-4.4	-2.8	-0.7
	WWWHKHGHGHGKHKNKGKKN	7.0	9.0	4.7	-3.7	-2.7	-0.6
	LLLNKKGKNKHKGHGHGHKH	5.9	4.9	4.0	-3.7	-2.0	-0.6
	WWWWHKHGHGHGKHKNKGKK	5.8	8.3	5.1	-2.8	-2.6	-0.6
	FFFFHKHGHGHGKHKNKGKK	7.7	8.1	3.9	-2.8	-1.8	-0.5
	LLLLHKHGHGHGKHKNKGKK	8.2	8.1	3.9	-2.8	-1.6	-0.5
	IIIIHKHGHGHGKHKNKGKK	4.8	5.9	3.4	-3.0	-1.5	-0.5
	LGKHKNKGKKNGKHNGWK	3.5	4.0	2.6	-4.9	-2.6	-0.8
	LLGKHKNKGKKNGKHNGWK	4.2	7.5	3.4	-4.2	-2.3	-0.7
	LLLGKHKNKGKKNGKHNGWK	5.2	7.2	3.7	-3.5	-2.0	-0.6
	AAAGKHKNKGKKNGKHNGWK	3.4	5.4	2.8	-5.1	-2.3	-0.7
	IIIGKHKNKGKKNGKHNGWK	4.8	6.6	3.3	-3.6	-1.9	-0.6
	FFFGKHKNKGKKNGKHNGWK	5.9	7.5	3.9	-3.4	-2.1	-0.6
	WWWGKHKNKGKKNGKHNGWK	7.1	9.8	4.1	-3.5	-2.7	-0.7

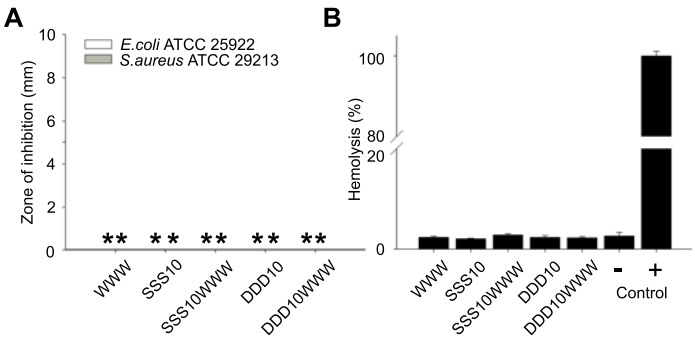
Fig. 1. *(A)* Antimicrobial activity and effects of various hydrophobic amino acid modifications (upper panel) and their location (lower panel). For determination of antimicrobial activities, *E. coli* ATCC 25922, *S. aureus* ATCC 29213 ($4x10^6$ CFU) or *C. albicans* ATCC 90028 ($1x10^5$) isolates were inoculated in 0.1% TSB agarose gel. Each 4 mm-diameter well was loaded with 6 µl of peptides at 100 µM. The zones of clearance correspond to the inhibitory effect of each peptide after incubation at 37 °C for 18-24 h (mean values are presented, n=3). (*B*) Effect of W- (left panel) and F-tag length (right panel) on GKH17 peptide activity (at 50 µM) against *E. coli* ATCC 25922 as assessed by RDA in the presence and absence of 0.15 M NaCl. *E. coli* ($4x10^6$ CFU) was inoculated in 0.1% TSB agarose gel. Each 4 mm-diameter well was loaded with 6 µl (100) µM of the indicated peptides. The zones of clearance correspond to the inhibitory effect of each peptide after incubation at 37 °C for 18-24 h (mean values are presented, n=3). "*" denotes no detectable clearance zone. *(C)* Effects of F-modified GKH17 peptides on HaCaT cells. The MTT-assay (upper panel) was used to measure viability of HaCaT keratinocytes in the presence of GKH17 and GKH17 peptides with variable F additions. In the assay, MTT is modified into a dye, blue formazan, by enzymes associated with metabolic activity. The absorbance of the dye was measured at 550 nm. Cell-permeabilizing effects of the indicated peptides (lower panel) were measured by the LDH based TOX-7 kit (mean values are presented, n=3). Results are shown in the absence (left) and presence (right) of 20% human serum. *(D)* Hydrophobic modification of antimicrobial peptides from HB-EGF (GKR22) and amphiregulin (PKR21) increases their bactericidal activity. Antimicrobial activity of the indicated peptides was assessed by radial diffusion assay (RDA) against *E. coli* ATCC 25922 at the indicated concentration (mean values are presented, n=3).

Fig. 2. *(A)* CD spectra of GKH17 variants in Tris buffer (left panel) and the same buffer supplemented by LPS (middle panel), or negatively charged DOPE/DOPG (75/25 mol/mol) liposomes (right panel). *(B)* Fluorescence spectra of W residues of GKH17-WWW (1 µM) in 10 mM Tris, pH 7.4, in the absence and presence of DOPE/DOPG liposomes. *(C)* Dynamic light scattering data on hydrodynamic diameter of GKH17-WWW and GKH17 at a peptide concentration of 10 µM in 10 mM Tris, pH 7.4. *(D)* LPS- and heparin-binding abilities of GKH17 and the indicated W-modified peptides. Peptides immobilized on membranes were incubated with radioiodinated LPS (*E. coli*), or heparin, washed, and radioactivity visualised.

Fig. 3. *(A)* Antimicrobial activity and effects of uncharged SSS10, negatively charged DDD10, their WWW-modified variants, and the WWW tripeptide. For determination of antimicrobial activities, *E. coli* ATCC 25922 or *S. aureus* ATCC 29213 ($4x10^6$ CFU) isolates were inoculated in 0.1% TSB agarose gel. Each 4 mm-diameter well was loaded with 6 µl of peptides at 100 µM. The zones of clearance correspond to the inhibitory effect of each peptide after incubation at 37 °C for 18-24 h (mean values are presented, n=3). "*" denotes no detectable clearance zone. *(B)* Hemolysis of the same peptides. Erythrocytes were incubated with peptides at 60 µM, and 2% Triton X-100 served as positive control. The absorbance of hemoglobin release was measured at 540 nm and is expressed as % of Triton X-100 induced hemolysis (note scale of y-axis).


A
E. coli ATCC 25922
S. aureus ATCC 29213
C. albicans ATCC 90028
Hydrophobicity
HKH17
AAAHKH17
PPPHKH17
YYYHKH17
VVVHKH17
IIIHKH17
LLLHKH17
WWWHKH17
FFFHKH17
HKH17
HKH17WWW
HKH17FFF
WWWHKH17
FFFHKH17
GKH17
GKH17WWW
GKH17FFF
WWWGKH17
FFFGKH17
B
− 0.15M NaCl
+ 0.15M NaCl
Zone of inhibition (mm)
No tag
W
WW
WWW
WWWW
WWWWW
LL-37
No tag
FFF
FFFF
FFFFF
LL-37
C
- Serum
+ Serum
Viability (MTT)
LDH release (%)
No tag
FFF
FFFF
FFFFF
+
-
Control
D
GKR22
GKR22WWW
PKR21
PKR21WWW
Concentration (μM)


A
Tris
LPS
DOPE/DOPG
[θ]/1000 (degree·cm⁻²)/dmol
0
-10
-20
-30
200 210 220 230 240 250 260
Wavelength (nm)
No tag
W
WWW
No peptide
B
Tris
DOPE/DOPG
Intensity (a.u.)
3000
2000
1000
0
300 320 340 360 380 400 420 440
Wavelength (nm)
C
GKH17
GKH17WWW
Intensity (a.u.)
35
30
25
20
15
10
5
0
0.1 1 10 100 1000
Size (nm)
D
No tag
WWW
WWWWW
LL-37
μg peptide
1
2
5
+ LPS
+ Heparin


A
B
E.coli ATCC 25922
S.aureus ATCC 29213
Zone of inhibition (mm)
10
8
6
4
2
0
**
**
**
**
**
WWW
SSS10
SSS10WWW
DDD10
DDD10WWW
Hemolysis (%)
100
80
20
0
WWW
SSS10
SSS10WWW
DDD10
DDD10WWW
-
+
Control