A Novel Target-Specific, Salt-Resistant Antimicrobial Peptide against the Cariogenic Pathogen *Streptococcus mutans*

Junni Mai[1,4], Xiao-Lin Tian[1], Jeffrey W. Gallant[3], Nadine Merkley[3], Zakia Biswas[3]

Raymond Syvitski[3], Susan E. Douglas[2,3], Junqi Ling[4] and Yung-Hua Li[1,2]*

Department of Applied Oral Sciences[1]

Department of Microbiology and Immunology[2]

Dalhousie University, Halifax, Canada

Institute for Marine Biosciences[3]

National Research Council of Canada, Halifax, Canada

Guanghua School of Stomatology[4]

Sun Yat-Sen University, Guangzhou, China

Supplemental Materials

***: Corresponding Author**

Mailing Address:

5981 University Ave. Rm5215

Halifax, Nova Scotia

Canada, B3H 3J5

Tel: 1-902-494-3063

Fax: 1-902-494-6621

E-mail: yung-hua.li@dal.ca

Table S1 Antimicrobial Activity of Pleurocidin Variants against *S. mutans* UA159

Name of peptide	Sequence of peptide	MIC (μg/ml)
NRC-1	GKGRWLERIGKAGGIIIGGALDHL*	32-64
NRC-2	WLRRIGKGVKIIGGAALDHL*	32
NRC-3	GRRKRKWLRRIGKGVKIIGGAALDHL*	4
NRC-4	GWGSFFKKAAHVGKHVGKAALTHYL*	2
NRC-5	FLGALIKGAIHGGRFIHGMIQNHH*	4
NRC-6	GWGSIFKHGRHAAKHIGHAAVNHYL*	4
NRC-7	RWGKWFKKATHVGKHVGKAALTAYL*	2
NRC-8	RSTEDIIKSISGGGFLNAMNA*	>128
NRC-9	FFRLLFHGVHHGGGYLNAA*	>128
NRC-10	FFRLLFHGVHHVGKIKPRA*	4-8
NRC-11	GWKSVFRKAKKVGKTVGGLALDHYL*	8
NRC-12	GWKKWFNRAKKVGKTVGGLAVDHYL*	8
NRC-13	GWRTLLKKAEVKTVGKLALKHYL*	16
NRC-14	AGWGSIFKHIFKAGKFIHGAIQAHND*	4-8
NRC-15	GFWGKLFKLGLHGIGLLHLHL*	16-32
NRC-16	GWKKWLRKGAKHLGQAAIK*	4-8
NRC-17	GWKKWLRKGAKHLGQAAIKGLAS	8-16
NRC-18	GWKKWFTKGERLSQRHFA	>128
NRC-19	FLGLLFHGVHHVGKWIHGLIHGHH*	16-32
NRC-20	GFLGILFHGVHHGRKKALHMNSERRS	>128
NRC-123	GWKDWFRKAKKVGKTVGGLALNHYLG	8
NRC-124	GIRKWFKKAAHVGKEVGKVALNACL	>128
NRC-125	GLKKWFKKAVHVGKKVGKVALNAYLG	4-8
NRC-126	GWRKWIKKATHVGKHIGKAALDAYIG	16
NRC-127	GCKKWFKKAAHVGKNVGKVALNAYLG	128
NRC-128	GIRKWFKKAAHVGKKVGKVALNAYLG	16-32

*: The peptides with *C*-terminal amidation. The MICs were determined in triplicate experiments using a modified method as described in Materials and Methods.

Table S2 Antimicrobial Activity of Fusion Peptides against Selected Bacterial Strains

	MICs (μM)			
Strains	NRC-4*	IMB-1	IMB-2	IMB-3
S. mutans UA159	1.0	2.2	2.8	44
S. mutans GS5	1.0	2.2	2.8	44
S. mutans NG8	1.5	2.2	2.8	44
S. mutans SmΔcomD	1.0	8.8	11.3	>44
S. sanguinis SK108	2.5	8.8	22.6	>44
S. gordonii DL1	2.0	8.8	22.6	>44

*: NRC-4 is the parent of the fusion peptides, IMB-1, -2 and -3.

Methods for NMR Spectroscopy

Sample preparation. Samples of peptides IMB-1, -2 and -3 were prepared for NMR analysis in 600 mL of 95/5 H_2O/D_2O (v/v) potassium phosphate buffer (pH 6.6) at final concentrations of 2.60 mM for IMB-1, 3.03 mM for IMB-2 and 2.32 mM for IMB-3. The samples were first lyophilized and resuspended in an aliquot of 1 M dodecylphosphocholine-D_{38} or DPC-D_{38} (Cambridge Isotope Labs, Andover, MA) to a final peptide:DPC ratio of 1:70. Samples were warmed to 60 ºC for 5 min, lyophilized and re-suspended in 600 mL of 95/5 H_2O/D_2O (v/v).

NMR analysis and data collection. ^{1}H, ^{13}C and ^{15}N NMR data were collected on a Bruker AVANCE 700 spectrometer at a frequency of 700.23 MHz with a triple resonance cryoprobe. NMR spectra were collected at 303.2 K with the water frequency centered on the carrier frequency. ^{1}H chemical shifts were referenced to 2,2-dimethyl-2-silapentane-5-sulphonate through calibration of the water resonance at different temperatures (Gottlieb *et al*, 1997, J Org Chem, 62:7512). ^{13}C and ^{15}N chemical shifts were indirectly referenced from the ^{1}H spectrum (Wishart *et al*., 1995, J. Biomol NMR, 6:135). Sequential assignments were made for the backbone residues using a phase-sensitive ^{1}H-^{1}H TOCSY (60 or 90 ms mixing time) or ^{1}H-^{1}H NOESY (200 or 120 ms mixing time). NMR data was processed using Bruker TopSpin 2.1 and analyzed using SPARKY 3 (Goddard TD and Kneller DG, University of California). Interproton distances were measured by the integration in the 200 ms ^{1}H-^{1}H NOESY. Interproton distances for proton pairs were calibrated using the nOe intensity for the signal between Ala Ha and Hb with a distance of 2.8 Å, and were classified into three groups (strong, medium and weak) corresponding to proton distance ranges of < 2.5, 2.5 – 3.5, 3.5 – 5.0 Å.

Structural determination. Structures of peptide IMB-1, IMB-2 and IMB-3 in DPC micelles were determined according to a simulated annealing protocol described previously (Syvitski *et al.*, 2005, Biochem. 44: 7282-7293) using the XPLOR 3.1 program (Schwieters *et al.*, 2003, J Magn Reson. 160: 65; Prog Nucl Magn Reson Spectrosc 2005, 48: 47).

NMR Diffusion. To determine if the peptides were associated with the DPC micelles, IMB-1, -2 and -3 were exchanged into D_2O by lyophilizing and redissolving in 1 mL of D_2O (99.99 atom %D), lyophilized and re-suspended in 600 uL of D_2O to minimize the intensity of the HDO peak and used for DOSY measurements after structural analysis. ^{1}H detected 2D DOSY data sets were recorded using stimulated-echo sequence with bipolar-gradient pulses and 32 t_1 blocks of 4 transients each. The 2D data sets were processed using Bruker TopSpin 2.1 and the t1 diffusion dimension was processed by fitting the rest of the F2 peaks to the equation:

$$A = A_o \exp(-Dg^2\gamma^2\delta^2(\Delta-\delta/3))$$

Where A is the observed Area and A_o is the reference intensity, D is the diffusion coefficient in m^2s^{-1}, γ is the ^{1}H gyromagnetic ratio (4257.7002 Hz G^{-1}), δ is the total length of the bipolar de-focusing re-focusing gradient pulses, optimized for each sample (0.0015 to 0.0013 s), Δ is the diffusion time (0.30 to 0.25 s), g is the gradient strength which was 32 exponential increments between 1.703 and 32.354 G cm^{-1}.

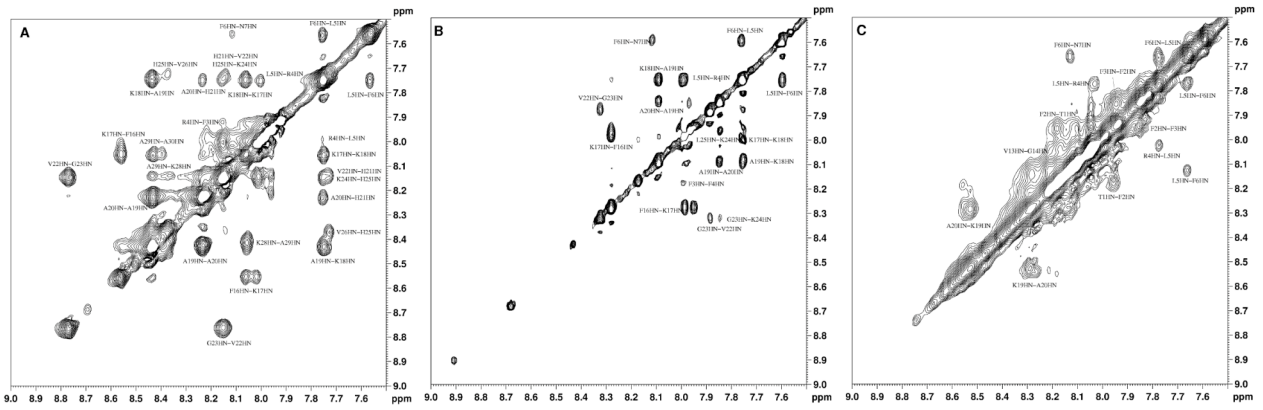

Fig. S1. H^N-H^N regions of the ^1H-^1H NOESY (200 msec mixing time) for IMB-1 (A), IMB-2 (B) and IMB-3 (C) in dodecylphosphocholine-D_{38} (DPC-D_{38}) micelles. IMB-1 (A) and IMB-2 (B) have reasonable chemical shift dispersion and the *NN*(i, i+1) connections, which suggest a helical structure, whereas considerably fewer *NN*(i, i+1) connections for IMB-3 indicates that it is unstructured and the H^N's are rapidly exchanging with water, which is consistent with a random coil structure.

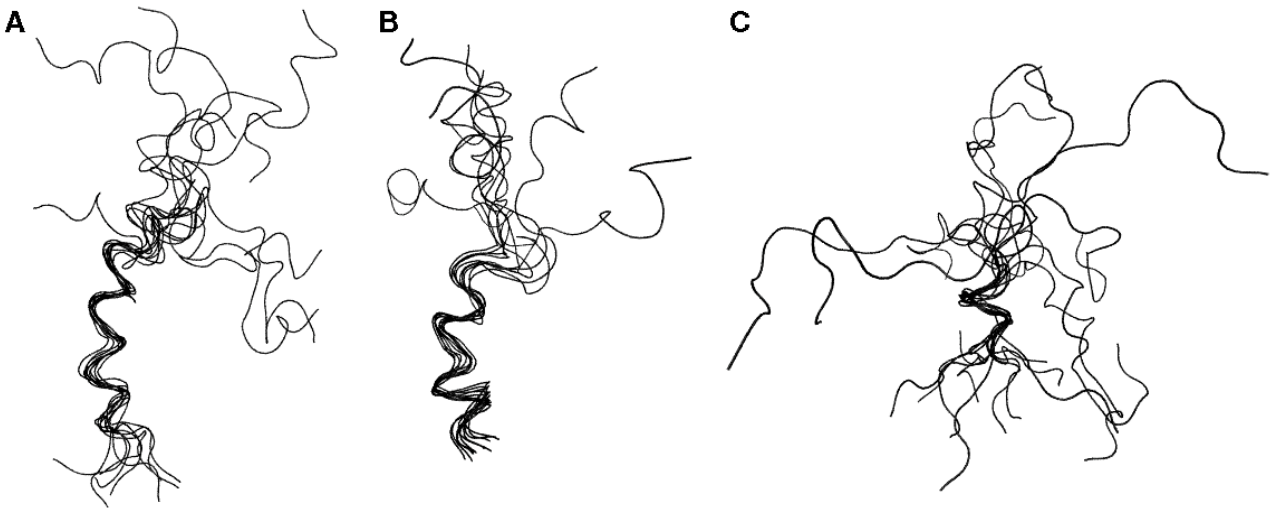

Fig. S2. An ensemble of the lowest energy structure of the fusion peptides IMB-1 (A), IMB-2 (B) and IMB-3 (C). Structured regions for each peptide are overlaid. IMB-1: residues 18-31 (r.m.s.d 1.33 to lowest energy structure) calculated from 182 nOe distance restraints; IMB-2: residues 12-23 (r.m.s.d 1.22 to lowest energy structure) calculated from 198 nOe distance restraints; IMB-3: residues 16-20 (r.m.s.d 1.02 to lowest energy structure) calculated from 114 nOe distance restraints. IMB-1 and IMB-2 form an amphipathic peptide with α-helical character with a flexible linker between the targeting and killing domains, whereas IMB-3 is random coil in DPC-d_{38} micelles.

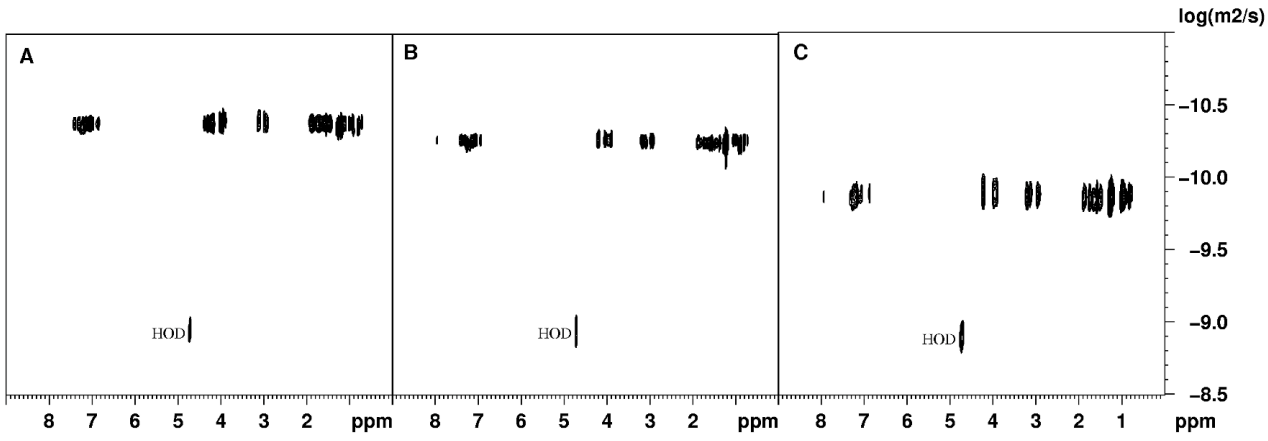

Fig. S3. DOSY spectra of the three fusion peptides in DPC micelles in which the F1 dimension represents the log(D) (where D is the self-diffusion constant). Both IMB-1 (A) and IMB-2 (B) have a similar diffusion constant but not to IMB-3 (C), suggesting the IMB-3 is not diffusing with the DPC-d_{38} micelles.

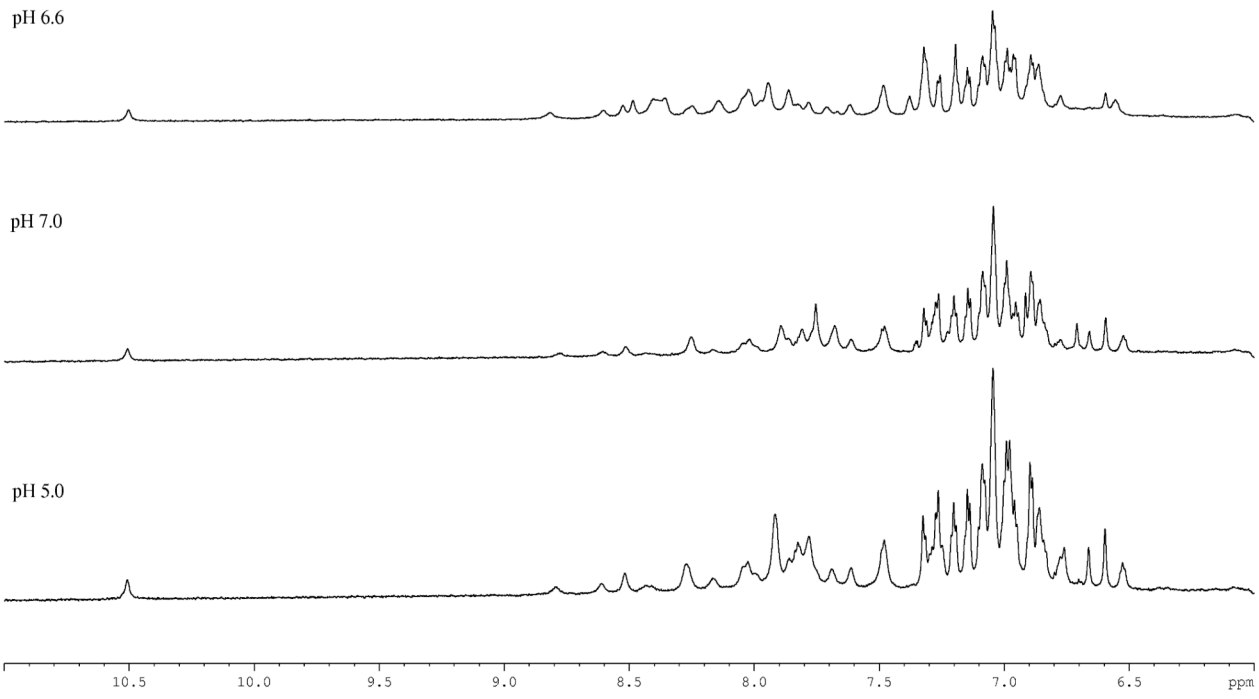

Fig. S4. 1-D ^{1}H NMR spectra of IMB2-H/S14 at pH 6.6, 7.0 and 5.0 in DPC-d_{38} micelles (peptide to lipid ratio 1:70). The peptide remains structured in a low pH environment.