Electronic Supplementary Material (ESI) for Nanoscale Advances.

Modulation of supramolecular self-assembly of an antimicrobial designer peptide by single amino acid substitution: Implications on peptide activity

Zhou Ye, Conrado Aparicio*

MDRCBB, Minnesota Dental Research Center for Biomaterials and Biomechanics, University of Minnesota, Minneapolis, Minnesota 55455, USA

*E-mail: apari003@umn.edu

Materials and methods

Peptide solution preparation

GL13K (GKIIKLKASLKLL-NH_2), D-GL13K with all D-amino acids (Gkiiklkaslkll-NH_2) and D-GL13K-I10 (Gkiiklkasikll-NH_2) were purchased from AAPPTec, LLC (Louisville, KY, USA) with purity >98%. GL13K analogues (A3, A5, A6, A7, A10, A11, and A13) and GL13-NH_2 were kindly donated by Professor Sven Gorr, University of Minnesota School of Dentistry. Stock solutions at a concentration of 10 mM were first prepared by dissolving peptides in deionized (DI) water. Borax-NaOH buffer solution at pH 9.6 was prepared by mixing 0.026 M borax solution with 0.1 M NaOH solution. To prepare the final peptide solution, 10 µl of peptide stock solution was added into 990 µl of buffer solution. All peptides were prepared in a pH 9.6 borax-NaOH buffer solution and incubated at room temperature until testing.

Circular dichroism (CD)

CD spectra of GL13K, GL13K analogues (A3, A5, A6, A7, A10, A11, and A13) and GL13-NH_2 were measured immediately after dissolving in the buffer solutions (0d) and after 1d, 2d, and 5d incubation at room temperature. The CD signal was obtained from a 200 µl peptide solution in a quartz cuvette with 1 mm path-length using a CD spectrometer (Jasco J-815, Easton, MD, USA). The measurements were averaged from 3 scans over a range from 260 nm to 190 nm with a data pitch of 1.0 nm, a scanning rate of 50 nm min^{-1} and a response time of 2 s. Background signal from pristine buffer solution was subtracted from all spectra.

Transmission electron microscopy (TEM)

After incubating for 1 day (for A11 analogue peptide) or 5 days (for all other peptides) at room temperature, 3 µl of GL13K or the analogue peptides solution was dispersed onto a negatively charged copper grid with a carbon film. The specimen was first washed with a droplet of DI water and immediately blotted with a filter paper twice. Then the specimen was stained with 5 µl of 0.75% uranyl formate for 45 s and blotted with a filter paper. After stained with 0.75% uranyl formate, the TEM sample was imaged using a FEI Tecnai G2 F30 (Hillsboro, Oregon, USA) at an accelerating voltage of 300 kV.

Estimation of secondary structure percentage

The secondary structure percentages of GL13K, GL13K analogues and GL13- NH_2 after 1 day incubation in the buffer solutions were estimated by the CDPro software (https://sites.bmb.colostate.edu/sreeram/CDPro/CDPro.htm, last accessed April 7, 2019) as described in detail elsewhere[1]. Briefly, the data were analyzed by three common methods in the software packages, SELCON3, CDSSTR and CONTIN/LL, with a reference set of 48 proteins (reference set 7 in CDPro software). The reported percentages were averages and standard deviations of the values estimated by the three methods.

Supplementary figures

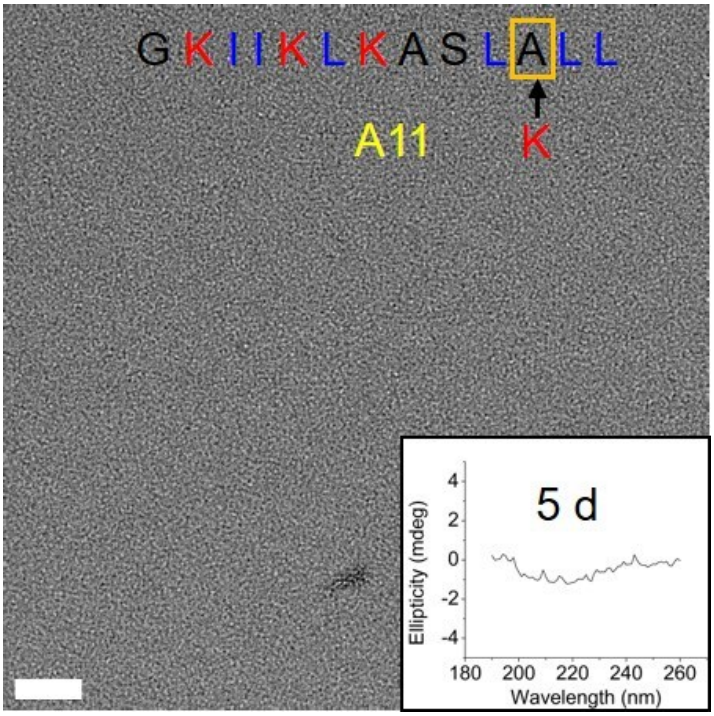

Figure S1. TEM micrographs and corresponding CD spectra of A11 after 5 day incubation. Scale bars are 50 nm.

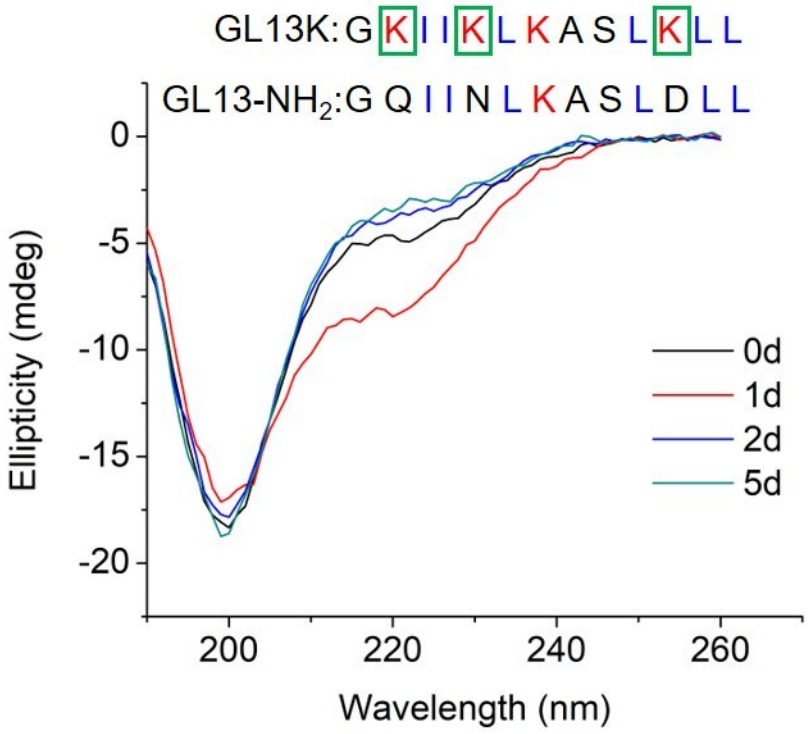

Figure S2. CD spectra of GL13-NH_2 in a pH 9.6 borax-NaOH buffer solution at room temperature. Percentage of unordered structure was estimated to be 72.4±14.1% and percentage of β-sheet was estimated to be 11.1±7.2% after 1 day incubation. The three amino acids that were substituted to lysine in the native GL13-NH2 peptide to derive the highly antimicrobial GL13K peptide are marked with green rectangles.

Reference:

1 Z. Ye, X. Zhu, S. Acosta, D. Kumar, T. Sang and C. Aparicio, *Nanoscale*, 2019, **11**, 266–275.