Sarkosyl-induced helical structure of an antimicrobial peptide GW-Q6 plays an essential role in the binding of surface receptor OprI in *Pseudomonas aeruginosa*

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Supporting Information

Methods

Paramagnetic relaxation enhancement (PRE) experiments

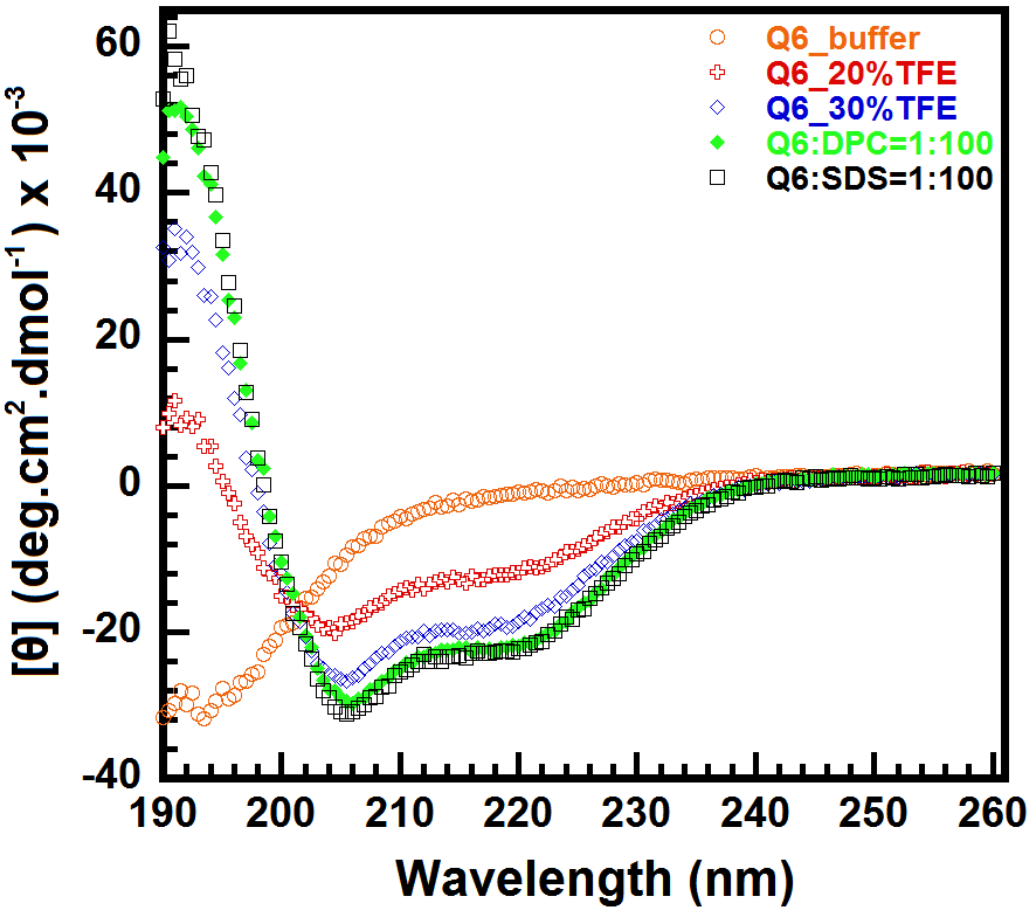
The paramagnetic relaxation enhancement (PRE) experiments were conducted with the addition of aliquots of Mn^{2+}, 5-doxyl-stearic acid (5-DSA), 12-doxyl-stearic acid (12-DSA), and 16-doxyl-stearic acid (16-DSA) in the NMR sample. Mn^{2+} ions solution (0.1M) was prepared by dissolving $MnCl_2 \cdot 4H_2O$ in 10 mM sodium phosphate pH 5.0 buffer. For paramagnetic relaxation enhancement effect of Mn^{2+} ions, GW-Q6 peptide was firstly mixed with DPC micelles (molar ratio = 1:100) and then vortexed for 3-5 min and incubating at room temperature for 10 min. After that, Mn^{2+} ions were added to reach the final concentration of 0.1, 0.5, and 1 mM in GW-Q6 (1.5 mM)-micelle solution, and equilibrated for 15 mins before recording ^1H-^1H TOCSY spectra. For paramagnetic relaxation enhancement effect, the DAS solutions were prepared in deuterated methanol (D_4-MeOH). The DSA was firstly mixed with DPC (in 10 mM sodium phosphate, pH 5.0) with a molar ration of 1:60. Subsequently, peptide GW-Q6, buffered in 10 mM sodium phosphate, pH 5.0, was added to the prepared DSA-DPC mixed micelle solution. The final concentrations of DSA, DPC and GW-Q6 were 2.5, 150, and 1.5 mM, respectively, and these NMR samples were equilibrated for 15 min before ^1H-^1H TOCSY spectra were recorded. All ^1H-^1H TOCSY spectra were acquired at 320 K with 2048 data points in t_2 and 320 points in t_1. The cross peak intensities of peptide GW-Q6 were measured with and without paramagnetic solutions and calculated based on established protocols [1].

Molecular Dynamics Simulation

The molecular dynamics simulations were conducted using the GROMACS software package, version 4.6.7. [2-4]. The GROMOSE96 force field parameters were employed for the peptide and solvent. The GROMOS united-atom parameter set, lipid.itp, and the pre-equilibrated micelles (65 DPC molecules) with 6305 water molecules were downloaded from Tieleman laboratory (http://moose.bio.ucalgary.ca). Our determined solution structure of GW-Q6 was used as the starting conformation for the simulation. GW-Q6 was initially placed at the DPC-water interface. The peptide-micelle system was placed in a 54 $Å^3$ periodic box of spc216 water. 47 chloride and 41 sodium counter ions were added as 0.1 mM electrolyte. GW-Q6 was positionally constrained and subjected to conjugated gradient minimization to eliminate interfering contacts [5]. The simulation was thermostatted at 310 K [6,7] and the temperature was coupled with Berendsen algorithm (coupling constant = 0.1 ps) [8]. The calculation of electrostatic interactions and the cutoff for van der Waals interactions were conducted with particle mesh Ewald algorithm [9]. The steepest decent algorithm was used for energy minimization down to a maximum gradient of 1000 kJ $mol^{-1}nm^{-1}$. NVT-ensemble state conduction was used for equilibrium, in which heavy atoms of peptide were positionally restrained by applying a spring constant of 1000 kJ $mol^{-1}nm^{-2}$. Additionally, all bonds were constrained with the LINCS algorithm [10]. After that, the isothermal-isobaric (NPT) equilibration was performed for 10,000 ps with Parrinello-Rahman algorithm (coupling constant = 0.5 ps) [11]. The position restraint used in NPT was also employed in NPT equilibration. Furthermore, the molecular dynamics simulation was carried out for 100 ns with an NPT ensemble.

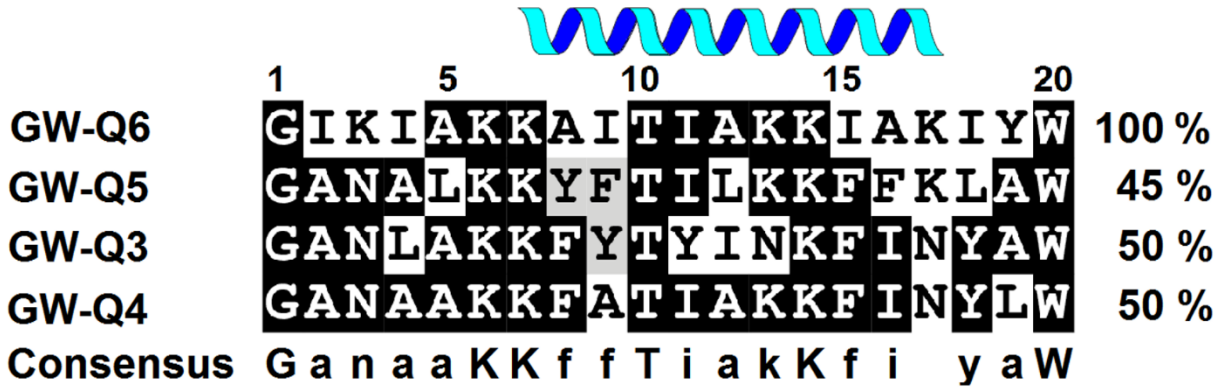
Figures

S1 Fig



S1 Fig. CD spectra of GW-Q6 in buffer, TFE, SDS and DPC.

S2 Fig



S2 Fig. Multiple sequence alignment of GW-Q6 and its analogues.

The multiple sequence alignment of GW-Q6 and its analogues (GW-Q3, GW-Q4 and GW-Q5) was conducted using the T-coffee algorithm and the on-line server ExPASy (www.uniprot.org/). The identical, conserved and semi-conserved residues are shaded in black, dark gray and pale gray, respectively. In the consensus sequence, capital and small letters are used to indicate those residues found in all or most of the sequences, respectively.

References

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