Supplementary Materials

Antimicrobial Peptide-grafted PLGA-PEG Nanoparticles to Fight Bacterial Wound Infections

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Experimental Section

Peptide purification and analysis

The crude peptide products were purified by reverse phase-high performance liquid chromatography (RP-HPLC) at preparative scale, on a Hitachi-Merck LaPrep Sigma system equipped with an LP3104 UV detector and an LP1200 pump, using an RP-C18 column (250 × 25 mm, 5 µm pore size) and an elution gradient using 0.05% aqueous trifluoroaceticacid (TFA) as solvent A and acetonitrile (ACN) as solvent B. The pure peptides fractions were collected, pooled, and freeze-dried on a Virtis Benchtop 9L freeze-dryer to afford the final peptides as fluffy white solids. Peptides' purity degrees were confirmed by analytical RP-HPLC using a Hitachi-Merck LaChrom Elite system equipped with a quaternary pump, a thermostatted automated sampler, and a diode array detector; analyses were performed with a reverse-phase C18 column (150 × 4.6 mm ID and 5 µm pore size, Merck) at a 1 mL/min flow rate using a 1–100% gradient of solvent B in solvent A, for 30 min, with detection at 220 nm. Peptides' molecular weights were confirmed by electrospray ionization-ion trap mass spectrometry (ESI-IT MS). Peptides' stock solutions were prepared at approximately 10 mg/mL in distilled water for accurate quantitation by microvolume spectrophotometry at 205 nm, using a Thermo Scientific™ NanoDrop™ One system and the quantitation method 31 that assumes an extinction coefficient (ε) at 205 of 31 mL·mg−1·cm−1 [1].

Results

Instrumental Analysis

Electrospray ionization-ion trap mass spectrometry (ESI-IT MS) analyses were run in a Thermo Finnigan LCQ Deca XP Max LC/MSn instrument using ion-trap quadrupole detection in positive mode.

Reverse phase high performance liquid chromatography (RP-HPLC) analyses were performed in a Hitachi-Merck LaChrom Elite system equipped with an L-2130 quaternary pump, an L-2455 diode-array detector (DAD) and an L220 thermostatted automated sampler. The samples were injected in a C18 column (125×4.0 mm ID and 5 µm pore size) with the elution gradient of 1 to 100% of B in A, using 0.05% aqueous trifluoroacetic acid (TFA) as solvent A and acetonitrile (ACN) as solvent B, and was run for 30 min at the flow rate of 1mL/min. The detection was performed at 220 nm.

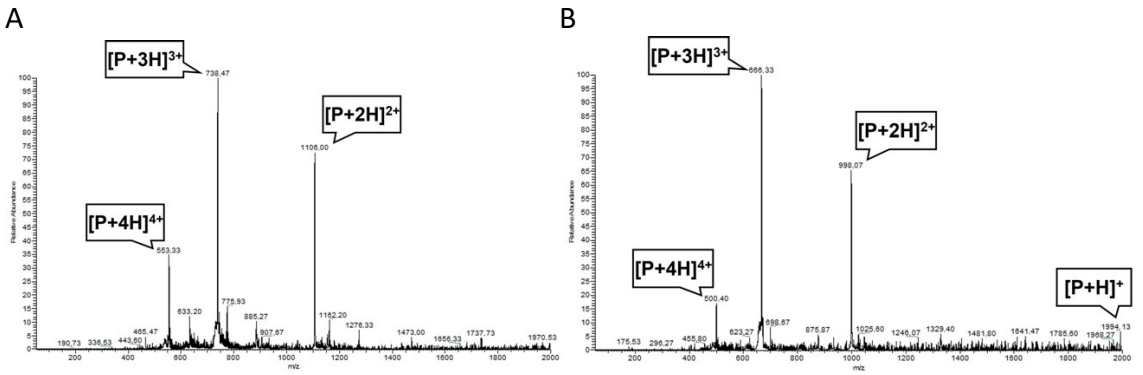

Figure 1- ESI-IT(+) mass spectrum of the (A) MSI-78 (4-20) and (B) MSI-78 (4-20)-Ahx-Cys. Multiply charged molecular ions of the target peptide, typically observed in ESI-IT MS analyses, are indicated as [M+nH]n+.

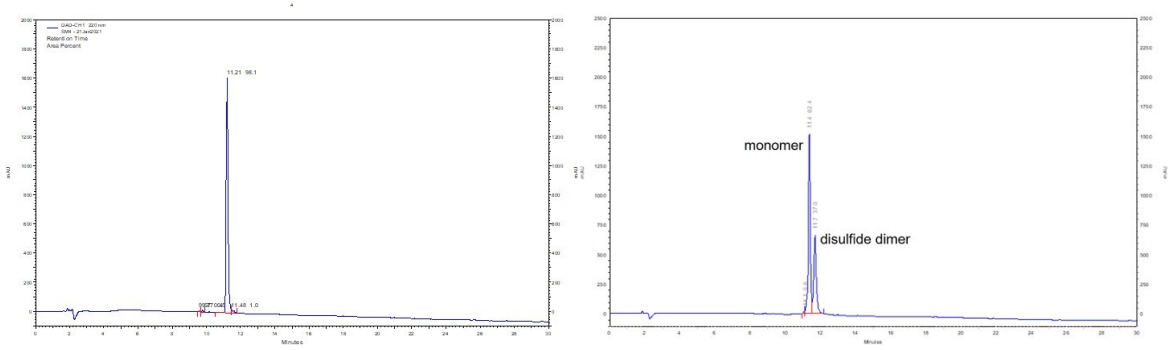

Figure 2 - RP-HPLC chromatogram of the (A) MSI-78 (4-20) and (B) MSI-78 (4-20)-Ahx-Cys.

References

1 Loughrey S, Mannion J, Matlock B. Using the NanoDrop One to Quantify Protein and Peptide Preparations at 205 nm, on Thermo Fisher Scientific, Wilmington, DE. http://tools.thermofisher.com/content/sfs/brochures/ND-One-Protein-and-Peptide-r16-01-18.pdf