Cecropin A-Melittin B Hybrid CA(1-7)M(2-9) Analogs with Improved Antibacterial Activity, Low Toxicity, and Good Protease Stability

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Introduction:

Cetropin A and Mellitin peptide hybrids have been a distinguished study for many decades. Cecropin A (1) compromises 37 amino acid and shows good antibacterial activity and is not toxic, however, it is too large to produce at a reasonable cost. Melittin is a 26-residue peptide that shows antibacterial activity, among others, but it is highly toxic to eukaryotic cells (2,3). Previously, it has been established that the hybridization of cecropin A and melittin has the optimal strategy with the combination of residues 1–13 of cecropin A (CA) followed by residues 1–13 of melittin (M), (4) which leads to an antibacterial peptide [CA (1–13)M(1–13)] of broad spectrum with a low hemolytic effect. However, this peptide sequence was still long (26 amino acid residues). Our group has managed to shortened the hybrid sequence even further to 15 amino acid residues, CA(1–7)M(2–9). Managed to synthesize this peptide with its analogs using solid phase peptide synthesis, characterized these peptides using reverse phase High Performance Liquid Chromatograpy (HPLC) and Liqiud Chromatography Mass Spectrometry (LC-MS). According to our findings CA(1–7)M(2–9) and its analogs have good antimicrobial activity, low hemolysis, and have good stability in tryptic digestion. These peptide properties solely depend on the construction of the hybrids, keeping in mind the fragment order, its amino acid composition, length, replacing of certain amino acids and the structure (linear/cyclic).

CA(1-7)M(2-9): H-KWKLFKKIGAVLKVL-NH_2

NaCme	Short name	Sequence	MW
CA(1-7)M(2-9)	CAM	H-KWKLFKKIGAVLKVL-NH_2	1770.3
CA(1-7)	CA	H-KWKLFKK-NH_2	976.3
M(2-9)	M	H-IGAVLKVL-NH_2	811.1
M(2-9)CA(1-7)	MCA	H- IGAVLKVL KWKLFKK-NH_2	1770.3
R_1-CA(1-7)M(2-9)	R_1-CAM	H-RWKLFKKIGAVLKVL-NH_2	1798.4
R_3-CA(1-7)M(2-9)	R_3-CAM	H-KWRLFKKIGAVLKVL-NH_2	1798.4
R_6-CA(1-7)M(2-9)	R_6-CAM	H-KWKLFRKIGAVLKVL-NH_2	1798.4
R_7-CA(1-7)M(2-9)	R_7-CAM	H-KWKLFKRIGAVLKVL-NH_2	1798.4
R_{13}-CA(1-7)M(2-9)	R_{13}-CAM	H-KWKLFKKIGAVLRVL-NH_2	1798.4
R-CA(1-7)M(2-9)*	R-CAM	H-RWRLFRRIGAVLRVL-NH_2	1910.4
O-CA(1-7)M(2-9)*	O-CAM	H-OWOLFOOIGAVLOVL-NH_2	1700.3
Cyclic C-CA(1-7)M(2-9)-C	Cyc-CAM		1974.6

Figure 1: The table above shows the amino acid sequence of CA(1–7)M(2–9) peptide hybrid and its analogs that were synthesized by our group. All peptides were synthesized using Rink-amide resin following Fmoc/tBu strategy in SPPS both in both automated and manual modes. After their cleavage all of them were purified by semi-preparative HPLC. The purities of the tested compounds ranged from 90–99% characterized by HPLC and LCMS.

Cyclization:H-CKWKLFKKIGAVLKVLC-NH_2

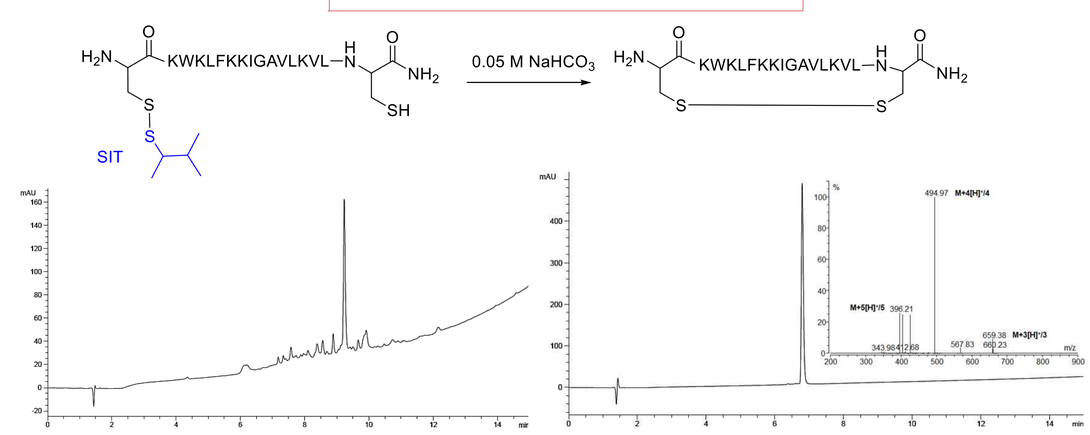

Figure 2: The disulfide cyclic version of CA(1–7)M(2–9) was obtained by adding a Cys residue to each end of the peptide and carrying out a chemoselective thiol–disulfide interchange using sec-isoamylmecaptan as protecting group of one of these residues. After peptide cleavage and precipitation, the crude peptide, was dissolved in a 0.05 M NaHCO3 solution to a dilution of 0.1 mM at a pH of approximately 8. The reaction was quenched by acidification to pH 2–3 using TFA and then required only a straightforward purification step in which a >99% purity was achieved.

MIC

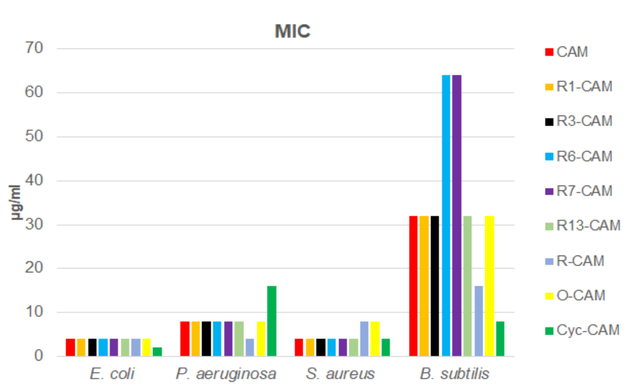

Figure 3: MIC (minimal inhibitory concentration) of peptides tested against gram negative (E. coli and P. aeruginosa) and Gram-positive S. aureus and B. subtilis) reference bacterial strains (ATCC strains). All nine peptides showed low MIC values against the four bacterial strains in a range from 2 to 64 µg/mL, the highest MICs being against B. subtilis.

% Hemolysis

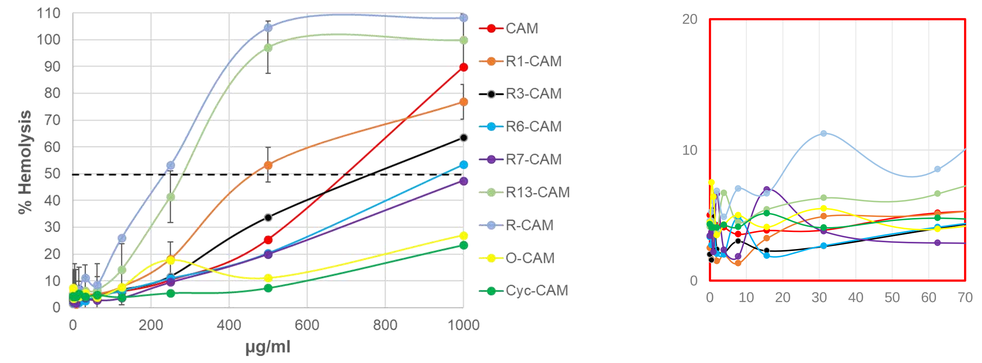

Figure 4: The graph is showing hemolytic activity of the peptides tested in Sheep Red Blood Cells. The R-CAM (high Arg content) showed the highest percentage of hemolysis as expected. The least hemolytic peptide was the disulfide-cycled version of CAM and O-CAM. However, of note, in the range of concentrations where the peptides showed antibacterial activity, the hemolysis value was below 10% for all of them, see the magnified part.

HC_{50} and Selectivity Index

	CAM	R_1-CAM	R_3-CAM	R_6-CAM	R_7-CAM	R_{13}-CAM	R-CAM	O-CAM	Cyc-CAM
SRBC (HC_{50} µg/ml)	690	450	750	935	>1000	280	230	>1000	>1000
SI *E. coli*	173	113	188	234	>250	70	58	>250	>500
SI *P. aeruginosa*	86	56	94	117	>125	35	58	>125	>63
SI S. aureus	173	113	188	234	>250	70	29	>125	>250
SI *B. subtilis*	22	14	23	15	>16	9	14	>31	>125

Figure 5: The table above is showing the R6-CAM, R7-CAM, O-CAM, and cyclic-CAM, had SI values that were significantly higher than that of the parent compound CAM. In contrast, as expected, the most hemolytic peptides, namely R13-CAM and R-CAM, presented the lowest SI values in comparison to CAM.

Trypsin Enzymatic Stability

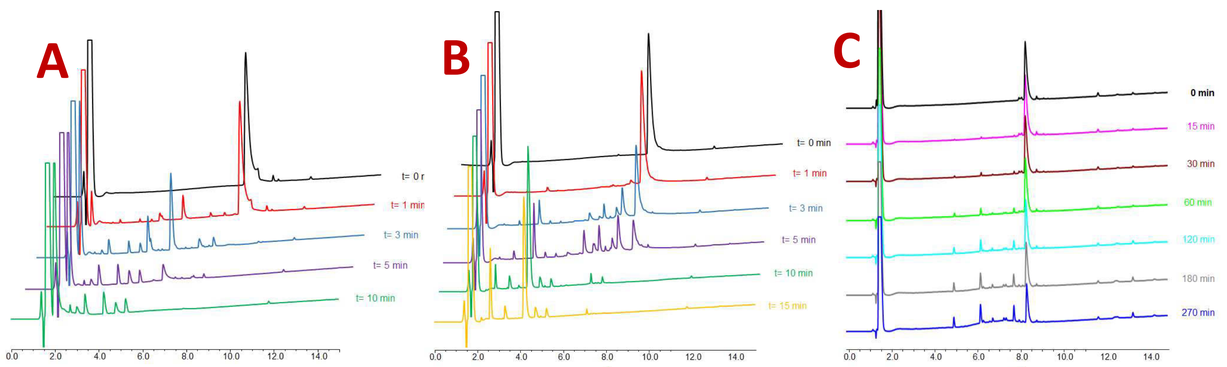

Figure 6: Time course of trypsin digestion of (A) CAM, (B) Cyc-CAM, and (C) O-CAM. In the case of linear CAM, there were no traces of the full-length peptide after 3 min. In contrast, for Cyc-CAM, the full-length peptide was still present at 5 min. The O-CAM showed the presence of 58% of the initial O-CAM in the medium after 4.5 h of tryptic digestion.

CONCLUSION:

- ✓ **CA(1-7)M(2-9):KWKLFKKIGAVLKVL** has good antibacterial activity and hemolysis, however they are easily digested by enzymes.
- ✓ **Cyclic C-CA(1-7)M(2-9)-C: CKWKLFKKIGAVLKVLC** has good antibacterial activity and hemolysis, has improved resistance to enzyme digestion
- ✓ **O-CA(1-7)M(2-9): OWOLFOOIGAVLOVL** has good antibacterial activity and hemolysis, has good resistance to enzyme digestion

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