# Interaction of Two Cecropin-Melittin Antimicrobial Peptides with Model Membranes: Calorimetric, Time Resolved Fluorescence Spectroscopy and Linear Dichroism Results

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Eukaryotic antibiotic peptides (EAPs) are important components of the non-adaptative immune system. These antimicrobial peptides have been widely studied for the past years as they may become an alternative to conventional antibiotic therapy, in view of the growing emergence of multi-resistant microbial strains. Substantial efforts have been directed to increase the potency and specificity of these peptides for pathogenic microbes while minimizing their cytotoxic effect towards eukaryotic cells.

One particularly successful approach in this direction is based on the synthesis of hybrid sequences derived from naturally occurring alpha-helical EAPs. CA(1-8)M(1-18), one of the most successful examples of the hybridation concept, showed improved antimicrobial activity relative to parent Cecropin A and at the same time greatly reduced the undesirable hemolytic effect of Melittin. Taking CA(1-8)M(1-18) as lead, a subsequent approach was to further reduce the size of the hybrid peptides retaining antimicrobial activity, as in CA(1-7)M(2-9). Both CA(1-8)M(1-18) and CA(1-7)M(2-9) have been extensively studied in terms of antimicrobial activity, but detailed biophysical studies are needed to fully understand their mechanism of action.

Therefore we have been studying the interaction of these two hybrids with liposomes by a variety of techniques, namely calorimetry (DSC and ITC), cicular dichroism, light scattering, SPR and fluorescence spectroscopy. The peptides were prepared by Fmoc/tBu solid phase synthesis methods, purified by reverse phase liquid chromatography and characterized by HPLC, amino acid analysis and MALDI-TOF mass spectrometry. LUV's from DMPC, DMPG and their 3:1 mixture were used as model membranes.

In the present report we will discuss the more recent results obtained by ITC, Time Resolved Fluorescence Spectroscopy and Linear Dichroism. The different techniques used provide us information on the energetics, partition and tertiary structure of the peptides as well as on their orientation relative to the liposome surface. The results obtained from the various techniques will be discussed together in an attempt to further understand the mechanism of action of these peptides.

Acknowledgements: Thanks are due to FCT for financial support to CIQ(UP), Unidade de Investigação 81, and for a Post-Doc grant to G.B (SFRH/BPD/5668/2001).