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Molecular Interactions and Assembly of the Antimicrobial Peptide Indolicidin in Layer-By-Layer Films

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Abstract – Indolicidin (ILPWKWPWWPWRR-NH_2) is a cationic antimicrobial peptide present in the cytoplasmic granules of bovine neutrofils. It consists of 13 amino acids with high tryptophan content and a broad spectrum of antimicrobial activity. Here we studied the molecular interactions of the nanobiocomposite indolicidin - polyelectrolyte PAMAM-sodium carboxylate obtained through the layer-by-layer (LbL) technique. We employed UV-VIS, fluorescence, and Fourier-transform infrared (FTIR) spectroscopies and atomic force microscopy (AFM) to show the molecular interactions and molecular self-assembly of this important antimicrobial peptide within nanostructured thin films. Taken together, the results increase knowledge of polypeptide-based thin film fabrication and will provide a reliable biomaterial-based system with potential applications as biocompatible and antimicrobial coatings, drug delivery systems and biosensors.

During the last two decades an increasing number of antimicrobial peptides have been discovered in animals and plants as well as bacteria[1]. These peptides act in vertebrates and invertebrates both as offensive and defensive agents by interacting with lipids and somehow disturbing the barrier properties of the membranes of the target cell. They possess a broad antimicrobial action, and in addition some of them also have antiviral and anticancer activity. One of the best characterized antimicrobial peptides is the indolicidin, a 13-residue amino acid sequence linear peptide with an unusual abundance of tryptophan. For being a well characterized system from biophysical and microbiological standpoints it is a good choice to explore it as a bioactive element for the development of novel nanostructured biomaterials for applications in nanomedicine. The aim of this study was to fabricate and to characterize nanocomposites made of indolicidin-polyelectrolyte PAMAM-sodium carboxylate. Such a system was fabricated using the layer-by-layer thin film technology with a solid planar substrate being dipped in polyelectrolyte and peptide solutions, respectively, both of them in 5 mM PBC (Phosphate-Borate-Citrate) buffer at pH 7.4. Measurements of UV-visible spectroscopy revealed an increase of absorbance with the number of bilayers indicating the deposition of the indolicidin-polyelectrolyte PAMAM-sodium carboxylate films on the solid substrate. Fluorescence spectroscopy indicated the interaction of indolicidin with PAMAM-sodium carboxylate films expressed by an appropriate emission spectrum. Fourier transform infrared spectroscopy (FTIR) analysis showed that the infrared transmission frequencies of the amide I bands of indolicidin were practically identical to those observed for indolicidin in model lipid membrane systems indicating that indolicidin conserves its functional conformation in the PAMAM-sodium carboxylate films. Atomic force microscopy (AFM) measurements gave insights on the morphology and topology of the films. This set of measurements point out to the possibility of developing nanoestructured functional composite biomaterials with potential nanobiotechnological applications.

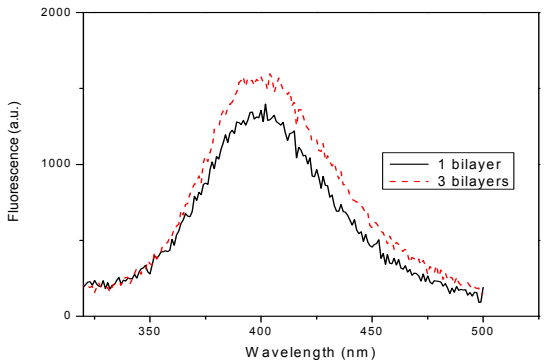

Figure 1: Fluorescence measurements showing the interaction of indolicidin with PAMAM-sodium carboxylate.

[1] Brogden, K. A., *Nature Reviews: Microbiology* v.3, p. 238-250, 2005.