Interaction of the Antimicrobial Peptide (AMP) Tritripticin and Its Analogues with Micelles Containing Negatively Charged Lysophospholipids. A Fluorescence Study

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INTRODUCTION: The 13-residue cationic AMP Tritripticin (TRP3, VRRFPWWWPFLRR), found in neutrophils, belongs to the cathelicidin family. TRP3 exhibits a wide spectrum against Gram-negative and Gram–positive bacteria, and fungi, and displays hemolytic activity. We investigated the interaction between TRP3 and two of its analogues and micelles consisting of zwitterionic lysophosphatidyl choline (LPC) and negatively charged lysophosphatidyl glycerol (LPG) or lysophosphatidic acid (LPA). OBJECTIVE: To investigate the role of lipid headgroup and peptide mutations on the interaction of TRP3 with model membranes. MATERIALS AND METHODS: TRP3, TRP3-LWL (W6 and W8 were replaced by L), and TRP3-WLW (W7 was replaced by L) were synthesized by solid phase synthesis. The micelles consisted of either LPC:LPG or LPC:LPA at a 4:1 molar ratio. Fluorescence spectra of the peptides were obtained in solution and in the presence of increasing micelle concentrations (pH 6.9 ± 0.1). Fluorescence quenching by water soluble acrylamide was examined in order to assess the depth of peptide penetration upon binding to the micelles. RESULTS: While the fluorescence of TRP3 and its analogues increased, the wavelength of maximum emission, λ_{max}, decreased with increasing concentration of both LPC:LPG and LPC:LPA, indicating that the peptides bound to the negatively charged micelles. Calculation of binding constants (K_b) indicated that the binding of TRP3-WLW was similar to that of the parent peptide. In contrast, TRP3-LWL bound considerably less to the micelles. A small decrease in binding was observed for LPC:LPA when compared to LPC:LPG micelles. Peptide binding was corroborated by the quenching studies. Calculated Stern-Volmer (K_{SV}) constants evinced a decreased accessibility of the peptides to acrylamide in the presence of micelles. Similar K_{SV} values were obtained for all peptide-micelle systems. CONCLUSION: The data indicated that TRP3 and its analogues interacted with micelles carrying negatively charged lysophospholipids and that the tryptophan residues in the bound peptides became less exposed to the aqueous environment.

Key words: tritrpticin, antimicrobial peptide, micelle

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