Interaction Bewtween TOAC^{0}-Labeled Histatin-5 And Biomimmetic Systems

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Histatins (Hsts) are His-rich antimicrobial peptides found in the saliva of primates. Hst-5 (DSHAKRHHGYKRKFHEKHHSHRGY) is known to have a remarkable action against *Candida albicans*, but Its mechanism of action is not known. Here we focused on the interaction between an Hst-5 analogue labeled with the paramagnetic amino acid 2,2,6,6-tetramethylpiperidine-1-oxyl-4-amino-4-carboxylic acid (TOAC) at the N-terminus (TOAC^{1}-Hst-5) and micelles and lipid vesicles of variable lipid composition. The micelles consisted of LPC, LPC:LPG (2:1), and LPG. Large unilamellar vesicles (LUV) were prepared so as to mimic the composition of *E. coli (*ePE:POPG:CL, 80:15:5)**,** *C. albicans* (POPC:ePE:PS:POPA:ERGO, 35:15:24:11:15), and erythrocytes (POPC:SM:ePE:COL, 26:22:7:45). The EPR spectra of TOAC^{1}-Hst-5 reported its binding to the model membranes. While the spectra in aqueous solution consisted of three narrow lines, due to fast tumbling, line broadening was observed upon binding due to the bilayer-imposed motional restriction. The peptide bound to a large extent to micelles and vesicles carrying a negative charge. In the presence of these model membranes two-component spectra were obtained, one due to peptide in the aqueous phase and the other due to the membrane-bound population. Spectral subtractions yielded the membrane spectra and showed that the peptide was strongly immobilized in bilayers mimicking *E. coli* and *C. albicans*. Estimation of the aqueous and membrane populations allowed calculation of partition coefficients (P). The values of P were found to be pH-dependent, decreasing with increasing pH, in a manner that paralleled the decrease of the peptide´s positive charge. The results point to the important role of electrostatic interactions for the peptide binding to lipid membranes.

Keywords: Antimicrobial peptide, histatin-5, EPR, micelle, lipid vesicle, model membrane.

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