Ion binding to the Antimicrobial Peptide (AMP) Histatin-5 (Hst-5). Effect on Peptides Conformational Properties

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The AMP His-5 (DSHAKRHHGYKRKFHEKHHSHRGY) belongs to the family of Histatins, low-molecular weight His-rich cationic peptides from salivary glands of humans and higher primates. Hst-5 has been extensively studied in view of its high fungicidal activity against *C. albicans*. Due to its low amphiphathic character, Hst-5 has been proposed to reach the cytoplasm in a non-lytic manner. Thus, Hst-5´s mechanism of action would differ from that of classical pore-forming peptides. However, although extensive studies have been done, little is known about this process. Furthermore, because of the high His content (almost 30%), Hst-5 presents several potential sites for interaction with metal ions. The aim of this work is to use CD, fluorescence, and EPR to study the effect of metal ions on conformational properties, interaction with model membranes, and functional properties of Hst-5 and its analogue containing the non-natural, paramagnetic amino acid TOAC (TOAC^0-Hst-5) to help elucidate Hst-5´s mode of action. Mn^{2+} EPR spectra showed that the ion interacts with the peptide. The number of coordinated ions seems to increase with increasing ion concentration. In studies with Cu2+, fluorescence spectra evinced quenching by increasing ion concentrations, in a saturating manner. These results were corroborated by CD data, where a band ascribed to aromatic residues was shifted and lost intensity upon addition of Cu2+. The data suggested that also in the case of Cu2+, complexes were formed with different ion:peptide stoichiometries. In further studies, we will investigate the role of ion binding in peptide-membrane interaction.
Keywords: Histatin-5; Antimicrobial peptide (AMP); Metal ions; Conformational properties.

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