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Glycine- and histidine-rich peptides derived from Shepherin I: study of candidacidal activity, metal-chelation properties and mode of action

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Introduction

Antimicrobial Peptides (AMPs) have been considered very promising candidates for the development of new drugs capable of killing antibiotic-resistant pathogens, a global public health problem. Thus, it is important to explore the structure, properties and mode of actions of this class of bioactive peptides [1]. Glycine- and Histidine-Rich AMPs (GRH-AMPs) are rare in nature and very little studied. This small group includes Shepherin I (Shep I) [1,2], which is found in the roots of the Korean plant *Capsella bursa-pastoris* (*Brassicaceae* family) as a cationic product of *shep*-GRP polypeptide proteolysis [3] and contains 28 amino acid residues (67.9% glycine and 28.6 histidine) with 7 repetitions of the ATCUN motif GGH. In 2014, we reported that Shep I and fragments are more efficient in inhibiting the growth of *Candida* cells in the presence of $ZnCl_2$ [4].

Objectives

The present study is part of our ongoing research focused on Shep I. We developed and studied amidated Shep I and new short truncated analogues aiming to better understand Shep I´s structure-antifungal activity relationship, mode of action, and ability to chelate Zn^{2+} and Cu^{2+} ions [1,4]. The results could also inform on potential applications of these peptides in the fight against two major global public health problems: candidiasis and neurodegenerative diseases related to Cu^{2+}-promoted amyloid plaque formation [5].

Experimental and Results

Peptide synthesis employed microwave-assisted solid phase at 60 ºC and customized protocols. The purified peptides presented high purity degrees and the predicted amino acid contents. As expected, Shep Ia, truncated analogues, and their corresponding N-carboxyfluorescein (FAM)-forms inhibited the growth of *C. parapsilosis* ATCC 22019 (MICs of 25-100μM), *C. krusei* ATCC 6258 (MICs of 6.25-100 μM) and *C. albicans* ATCC 90028 (MICs ≥ 6.25 μM). Some of them also killed the fungal cells (MFCs of 6.25-100 μM) and became more potent in the presence of Zn^{2+} ions, confirming our previous observations [4]. Experiments using fluorescence spectroscopy, special metal-chelators and ESI-MS showed that the active FAM-peptides bind Cu^{+2} and Zn^{+2} ions with molar ratios of 1 Peptide:2 Cu^{2+} ions and 1 Peptide:10 Zn^{2+} ions. To speculate possible mode of actions for such efficient candidacidals, phase contrast and epifluorescence images were obtained using them and very simplified models of *Candida* plasma membrane (Giant Unilamellar Vesicles produced by *natural swelling* [6]); the results showed that the active fluorescent peptides strongly interact with the vesicles and penetrate and/or disrupt them after exposure of 20 min. *In vitro*, Shep I and new truncated analogues also competed with synthetic β-amyloid peptides for Cu^{+2} ions and prevented the formation of reactive oxygen species [7], findings highly suggestive of potential ability to interfere in Cu^{+2}-assisted amyloid plaques formation typical of neurodegenerative diseases. Preliminary *in vivo* experiments using FAM-octapeptide

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showed penetration not only into *Candida* cells into *Caenohabditis elegans*, used as used as model organism for Blood Brain Barrier penetration [8].

Conclusions

C-amidated Shep I, some of the new short analogues and their corresponding FAM-forms are efficient candidacidals, potent chelators of divalent metal ions, and/or cell-penetrating peptides. Such functional versatility suggests that these Shep I-derived peptides find applications in science, human health and nutrition, agriculture, chemical and environmental activities.

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