*Pisum sativum* defensin 1 induces fungal death by an apoptosis-independent mechanism.

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Plant defensins are positively charged, cysteine-rich, 4-5 kDa peptides with antifungal activity. Defensins are usually non-toxic to mammalian cells, highlighting their potential application as anti-microbial drugs. We have previously characterized *Ps*₁, a 5.4 kDa defensin isolated from the pea *Pisum sativum*. In a 20 µM concentration, *Ps*₁ leads to 95% and 80% growth inhibition of *Candida albicans* and *Aspergillus nidulans*, respectively. It was also showed that a glucosylceramide found in fungi membrane, CMH, is important to *Ps*₁ inhibitory activity, as *C. albicans* cells mutant for ceramide synthase are 25 % less susceptible to the peptide than the parental yeast strain. In this work, we investigated the mechanisms involved in the cell death provoked by *Ps*₁. We characterized several markers of apoptosis, such as the accumulation of reactive oxygen species (ROS), induction of metacaspases and presence of DNA strand breaks. Although *A. nidulans* hyphae incubated with 20 µM *Ps*₁ exhibited cell wall and membrane injury, we weren’ t able to detect any apoptosis-like phenotype by TUNEL assay or ROS induction. We also analyzed whether *Ps*₁ caused necrosis in fungal cells using the Propidium Iodide (PI) internalization assay. We observed 12% PI positive cells after *Ps*₁ treatment, in contrast to 1.5% in the absence of the peptide. Further experiments using mutants in CMH synthesis and in key components of necrosis pathways will be explored. Unraveling the cellular mechanisms by which plant defensins lead to fungal death will bring new insights to the development of novel antimycotics. Financial support: CNPq, FAPERJ and FAPESP.
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