SITE-DIRECTED MUTAGENESIS OF PSD1: UNDERSTANDING ITS MECHANISM OF ACTION

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*Ps*d1 is a 46 amino acid plant defensin isolated from pea which exhibit antifungal activity. Its structure is characterized by a cysteine-stabilized α/β motif as determined by NMR. Hydrodynamic properties of *Ps*d1 revealed that the domains Thr9-Asn17 and His36-Trp38 display large conformational fluctuations. A consistently shift perturbation in the 15N-HSQC spectra of *Ps*d1 of these regions was also observed in the presence of vesicles doped with a cerebroside isolated from *Fusarium solani*. To demonstrate the importance of these regions four *Ps*d1 recombinant mutants (G12K, G12E, H36K, and H36E) were obtained in *Pichia pastoris*. The cDNA containing the corresponding mutations were synthesized, inserted into the pPIC9 vector and the transformation of *P. pastoris* were achieved. Ten *P. pastoris* His^- selected colonies were cultured in 5 mL BMG medium for 24h at 30°C, centrifuged and resuspended in 5 mL of BBS medium in order to induce protein expression for 72 h at 30°C by addition of 0.7% methanol at every 24h. SDS-PAGE and MALDI-TOF assays confirmed the secretion of 5kDa recombinant peptides. One colony of each *Ps*d1 mutant presenting the highest protein expression level, as confirmed by SDS-PAGE and protein content, was chosen for large scale protein production. The site directed mutants will be important for the comprehension of the relationship between structure and function of antifungal *Ps*d1 defensin. Supported by: ICGEB, CNPq, FAPERJ.