FUNCTIONAL AND STRUCTURAL CHARACTERIZATION OF TWO DEFENSINS FROM SUGARCANE: Sd1 AND Sd5

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Plant defensins are proteins of approximately 5 kDa presenting a cysteine-stabilized α/β motif. Despite the similarity in three-dimensional folding they can present distinct activities, including antifungal, antibacterial or inhibition of alpha-amylases. The exact structure/function motif is not known. The target of our work was Sd1 and Sd5 plant defensins selected from SUCEST-FAPESP project database. They were obtained by heterologous expression in *E. coli* BL21(DE3) and purified by HPLC. Antifungal assays showed that Sd5 was active against *Neurospora crassa* and *Aspergillus niger* (IC_{50}~5 µM). Both proteins did not present antibacterial activity. The α-amylase inhibitory activity, monitored by isothermal titration calorimetry (ITC), showed that Sd1 is capable to inhibit saliva, fungus and bacteria alpha-amylases, while Sd5 had no effect. In order to determine the solution structure of Sd5 we performed two and three-dimensional spectra. The complete assignments of backbone and side chain hydrogen atoms resonances were obtained. CSI analysis showed very similar secondary structure to other defensins, but heteronuclear NOE data suggested a flexible C-terminal region that differ form other defensins. We are now performing the NOE analysis to proceed in the calculation of Sd5 structure.

Keywords: NMR, plant defensin, calorimetry

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