The importance of the Gly12 and His36 aminoacid residues for *Ps*d1's antifungal activity.

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Plant defensins are proteins of low molecular weight (~ 6 kDa), rich in cysteine residues that present a distinctive antifungal and/or antibacterial spectrum of activity, suggesting their application as natural antimicotics and/or antibiotics. The antifungal peptide *Ps*d1 isolated from pea seeds exhibits a cysteine stabilized a/ß motif structure linked by three loops as determined by two-dimensional NMR. The presence of the monohexosylceramide (CMH) isolated from the fungus *Fusarium solani* induced pronounced changes in the chemical shift of some aminoacids presented in the region of the first (Thr9-Ala18) and of the third (His36-Trp38) loops which are coincident with those that exhibit conformational fluctuations suggesting that CMH may be the primary target of the fungus sensibility to *Ps*d1. To demonstrate the importance of these two regions for its antifungal property four site directed mutants: *Ps*d1Gly12Lys, *Ps*d1Gly12Glu, *Ps*d1His36Lys, and *Ps*d1His36Glu were successfully produced by the *Pichia pastoris* yeast. After protein purification their antifungal activity were tested against *Fusarium solani* in micro plate assays. At concentration of 5 µM the four mutants inhibited less than 20% the growth of *Fusaruim solani* in contrast with the growth inhibition greater than 40% exhibited by the native protein. The association and dissociation curves of protein with vesicles of PC:CMH (7:3 M/M, 50 nm diameter) bound to a L1 chip were obtained by surface plasmon resonance (SPR) assays. A response unit (RU) of 95 was observed to *Ps*d1 while RUs of 15, 50, 47 and 9 were observed for *Ps*d1Gly12Lys, *Ps*d1Gly12Glu, *Ps*d1His36Lys, and *Ps*d1His36Glu, respectively. These results suggested that both residues are important to the interaction of *Ps*d1 with fungal membrane/wall and consequently to its antifungal activity. Supported by: FAPERJ/CNPq.