Molecular homology and docking of a natural cysteine-proteinase and cystatin from cacao

Cardoso, THS[1], Andrade, BS[2], Santiago, A.S[1], Dias, CV[1], Koop, DM[1], Micheli, F[1,3], Góes-Neto, A[4], Cascardo, JCM[1†], Alvim, FC[1], Pirovani, CP[1]

[1]DCB/UESC,, [2]UESB-Bahia [3]Cirad-BIOS, UMR DAP, Montpellier, France

Cysteine-proteinases and cystatins are proteins related with defense mechanisms against pathogen attack, and involved in the endogenous regulation of programmed cell death. Genes of both proteins were encountered in *T. cacao – M. perniciosa* interaction cDNA library. Bioinformatics analysis revealed that the cysteine protease contains an amino terminus signal peptide with probable cleavage site after the 19th aa followed by a conserved auto-inhibitory domain of 56 aa and a catalytic domain of 218 aa, characteristic of the C1 peptidase family. The cystatin (205 aa) contains a conserved phytocystatin motif and a site inhibitory of proteinase. Modeling homology of both proteins using Modeller9 v.8 software showed that the cysteine-proteinase needs to lose its inhibitory domain to become active and leave the catalytic cleft exposed to substrate. Molecular docking using ClusPro 2.0 software revealed the *in silico* interaction between the enzyme and inhibitor from cacao. This result was confirmed by experimental capture of the proteinase using the cystatin followed by the identification of the captured protein by mass spectrometry. Thus, we evidenced by *in silico* and *in vitro* analysis that these two proteins interact, balancing their performance at cellular level in response to different stimuli such *M. perniciosa* infection.

Keywords: cysteine-proteinase, cystatin, modeling, docking, protein capture.

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