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A pipeline to study structural interactions among *Spodoptera frugiperda* serine proteinases and plant proteinase inhibitors

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Plants produce proteinase inhibitors (PIs) as a form of defense against herbivore insects. These molecules bind to insect midgut proteolytic enzymes, rendering them inactive by competitive inhibition. However, an increasing number of studies have shown that some insects are able to bypass this chemical protective barrier. Previously, our laboratory assembled a *Spodoptera frugiperda* cDNA library, which enables us to identify eight chymotrypsin sequences. We propose here a computational biology pipeline to identify and analyze possible structural determinants that could explain some level of insensitivity by *S. frugiperda* serine proteinases (SPs) against plant PIs observed in a real time PCR experiment. First, using protein structure modeling based on sequence similarity, we propose up to 30 models with different conformation assembling for each chymotrypsin previously identified. Secondly, all proposed models are to be refined by molecular dynamics, and, according to the MD index, the best model for each one is to be indicated. The next step is the protein – inhibitor complexing. All the best models are complexed by structural alignment with 13 Bowman-Birk PIs structures available at PDB filtered by its Resolution (< 2.2 A), R-Value and if it is complexed with a serine-proteinase. To increase the reliability of the process for complex formation we wrote two python scripts, dock_in and dock_out, that interact with Pyrosetta and perform a molecular docking with refinement by molecular dynamics. The Pyrosetta results were parsed by dock_pars script. A perl script that generates a matrix with all Rosetta full-atom scoring functions. Based on Total score, RMSD, Van der Waals net attractive energy, Van der Waals net repulsive energy, Solvation and Electrostatic potential we were able to propose an affinity rank among all chymotrypsin used in this study. The results from computational biology analyses indicates that Chymotrypsin nº 09 (SfChy9) was one of the most advantageous for *S. frugiperda*, since this protein present lower affinity for all inhibitors tested. Real time PCR indicated an increased expression of SfChy9 when compared to the other enzymes. Future analyses might take place in order to evaluate the correlation between PIs insensitivity and expression activation or deactivation, since parallel studies in our laboratory has pointed to this correlation might not be linked to an expression feedback.
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