Molecular and Functional Characterization of CmPI-II a "Non-classical" Kazal-type Inhibitor: Molecular Dynamics Simulations and Experimental Evidences

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Proteases and its inhibitors are subjects of intense research for development of new drugs and the study of protein-protein interactions. Recently, we reported the isolation and characterization of the *Cenchritis muricatus* protease inhibitor (CmPI-II), a Kazal tight binding inhibitor of serine proteases: trypsin, human neutrophil elastase (HNE), subtilisin A and pancreatic elastase. CmPI-II (UNIPROT: P84755) is an unusual inhibitor of HNE, since it has an arginine residue at P1 site, contrary to the HNE preferences for small, hydrophobic residues in this position. Here, we present three-dimensional models of CmPI-II in complex with HNE to obtain a rationale of its behaviour, and to design CmPI-II mutants with an increased specificity against HNE. Molecular dynamics simulation results suggested that differences in specificity between arginine and hydrophobic residues at P1 site of CmPI-II correlates with side-chain accommodation into S1 enzyme pocket, rather than final amino acid residue interactions. Remarkable, accommodation of CmPI-II Arg12 in S1 pocket could be related with increased reactive loop flexibility due to unusual position of the Cys^{I}-Cys^{V} disulfide bridge. Furthermore, the presence of a tryptophan residue at CmPI-II P2´ site also seems essential for HNE inhibition. Site-directed mutagenesis and kinetic studies corroborated *in silico* analyses (Supported by IFS (Stockholm, Sweden) and OPCW, The Hague, Netherlands, through of the grants F-2927/1, F-2927/2, F-3772/1 and F-3342/1, UNU-BIOLAC (ONU, Japan), FAPESP, CNPq and CAPES).