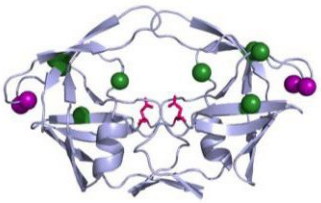
Effect of novel hinge insertions on drug binding and structurally stability of the South African HIV-1 subtype C protease

Sheik Ismail, Z.[1] and Sayed, Y.[1]

[1]Protein Structure-Function Research Unit, The University of the Witwatersrand, Johannesburg, South Africa, 8000



A ribbon image depicting a homology model of L38↑N↑L^{+4} protease. The hinge region double insertion of Asparagine and Leucine are shown as purple spheres, with the accompanying subset of background mutations shown as green spheres. (PDB ID: 3u71.)

Introduction: Acquired immunodeficiency syndrome (AIDS) caused by the pathogenic Human Immunodeficiency Virus (HIV) remains a major global health concern, particularly in sub-Saharan Africa with the HIV-1 subtype C protease accounting for approximately 95% of infections. A South African HIV-1 subtype C protease (HIV-1 C-SA PR) carrying a subset of polymorphisms (K20R, E35D, R57K, V82I) and a double insertion of asparagine and leucine at position 38 in the hinge regionof the protease (L38↑N↑L^{+4}) was isolated from a drug naïve-infant. The aim of this study was to determine how these novel hinge region insertions affect drug binding thermodynamics, structural changes and conformational stability of the protease. This was done by creating a mutant containing only the double insertions in the hinge region of the protease, L38↑N↑L^{-4}.

Methodology: The wild type (WT) and mutated L38↑N↑L^{-4} proteases were successfully over- expressed and purified using ion exchange chromatography. The purity of both proteins was assessed using SDS-PAGE to ensure homogeneity for downstream experiments. Isothermal titrationcalorimetry (ITC) was used to determine the amount of correctly folded active protein as well as the binding affinity of the protease inhibitors (PIs) atazanavir (ATZ), darunavir (DRV), ritonavir (RTV) and saquinavir (SQV). Additionally, differential scanning calorimetry (DSC) was performed to assess the structural stabilities of the proteases in the absence and presence of these four PIs. *In silico* studies including molecular dynamic simulations and induced-fit molecular docking were performed on the
WT, L38↑N↑L^{-4} and L38↑N↑L^{+4} proteases in order to provide information of the flexibility and dynamics of the proteins.

Results: ITC showed that both the WT and L38↑N↑L^{-4} proteases had >75% active protease and displayed moderate binding (μM) to the aspartyl protease inhibitor acetyl pepstatin. The binding affinity of PIs for the WT and mutant was 7.9 nM and 16.2 nM for ATV, 1 nM and 49 nM for RTV and 107 nM and 133 nM for SQV. The binding affinity for DRV was 1 nM for both the WT and mutant protease. The melting temperatures for the apo WT and mutant were 63.9 °C and 68.9 °C, respectively. The WT melting temperatures for ATV, DRV, RTV and SQV were 88.2 °C, 92.6 °C, 86.3°C and 87.1 °C, respectively. The mutant produced two melting temperatures for each of the PIs: specifically, – 74.3 °C and 84. 3°C for ATV, 75.5 °C and 85.1 °C for DRV, 73.8 °C and 84.3 °C for RTV and 70 °C and 80 °C for SQV. Molecular dynamics simulations studies indicated that RMSD values for all three proteases were in a 1-3 A° range while RMSF values showed a 20% change of the mutants compared to the WT.

Discussion and Conclusion: Insertions in the hinge region decrease the binding affinity of the proteasetowards ATV, RTV and SQV suggesting that they may play a role in the ability of the protease to bind some PIs. The apo L38↑N↑L^{-4} protease had a higher melting temperature than the WT suggesting the mutant is structurally more stable. However, when complexed with PIs the mutant produced lower melting temperature values compared to the WT indicating that in a PI-complexed state the mutant is less stable. The MD simulations show that the flap regions of the mutants are more stable than the WT indicating the role of these insertions in stability. Taken together the data suggest that the double insertion in the hinge region plays a role in altered binding mechanisms and structural stability of the protease providing novel data on drug dynamics which may affect HIV patient treatment and drug response.

References: WHO 2016. Consolidated guidelines on the use of antiretroviral drugs for treating and preventingHIV infection: recommendations for a public health approach. *WHO Press,* Geneva, Switzerland.

Keywords: HIV, protease, drug binding interactions, structural stability