Structural and functional studies of South African HIV-1 subtype C proteases: impact on protein dynamics, stability, and drug binding to the wild type and mutants

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Abstract

Human immunodeficiency virus (HIV) continues to present a significant challenge to the health of millions of individuals globally. In the HIV cell cycle, three enzymes, reverse transcriptase, integrase, and protease, are involved in viral maturation. Of the three enzymes, we focused on the protease as a drug target in antiretroviral therapy. HIV protease is a homodimeric aspartic protease (D25) that naturally contains 99 amino acids per monomer. We used the wild type and two mutant proteases [triple mutant inactive form (I13V, I62V, and V77I; HP3)] and a double insertion mutant (L38↑H↑L) located in the hinge region, respectively, for structural and functional characterization using both in-silico and wet lab experiments. Each protease was expressed and purified from Escherichia coli BL21 (DE3) pLysS competent cells. The cells were transformed separately with pET-11a vectors, each containing the protease gene insert mentioned earlier. The purified proteins were subjected to biophysical and biochemical characterization, followed by crystallization trials. The wild-type and HP3 protease structures were solved at 2.1 Å and 1.8 Å, respectively, using X-ray crystallography. The crystal structure of the mutant (HP3) revealed that the location of the above-mentioned mutations and their effect on the hydrophobic sliding mechanism may be crucial in their role in resistance. Isothermal titration calorimetric studies of some FDA-approved drugs are discussed. In addition, molecular docking of one novel and 10 FDA-approved drugs was performed with saquinavir (SQV) showing the best affinity towards the target proteases. The novel drug also exhibited good affinity towards the proteases when compared to second-generation protease inhibitors such as lopinavir (LPV) and darunavir (DRV), which are used in clinical practice to treat HIV. The simulations of all proteases in the apo and selected complex forms with SQV and novel drugs were performed and are discussed.