Double amino acid insertion in the hinge region of HIV-1 South African subtype C protease displays enhanced catalytic properties relative to the wild-type protease

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The development of mutations in the human immunodeficiency virus (HIV) genome has become a crucial factor in limiting antiretroviral therapy for HIV/AIDS treatment. By the end of 2020, approximately 37 million people globally were infected with HIV. South Africa, in particular, bears the brunt of the HIV-1 subtype C epidemic. The HIV protease is a homodimeric protein that naturally contains 99 amino acids per monomeric subunit. The protease is vital in the HIV life cycle because it cleaves Gag and Gag-Pol precursor polyproteins into proteins necessary for viral assembly, maturation, and infection. Herein, we performed a comparative study between the HIV-1 subtype C protease (wild- type) and a protease containing a double amino acid insertion (histidine and leucine at codon 38), L38↑H↑L. Far-UV circular dichroism and size exclusion chromatography results indicated that the L38↑H↑L protease is a predominantly beta-sheeted protein with a homodimeric molecular weight of 22kDa which are consistent with characteristics pertaining to the wild-type protease. The L38↑H↑L protease displayed a 93% decrease in K_M and 29% reduction in the catalytic turnover number, k_{cat}. The L38↑H↑L protease was also found to be 11 times more efficient in catalyzing the hydrolysis of the fluorogenic substrate. The data suggests that amino acids in the hinge region of HIV-1 proteases indirectly affect enzyme catalysis and substrate specificity but has no effect on the conformational stability of the L38↑H↑L protease.