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EXPRESSION OF AN ACTIVE HIV-1 SUBTYPE C PROTEASE

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ABSTRACT

Similar to other retroviruses, host cell infection with HIV-1 requires three key steps in the viral replication process: Firstly, reverse transcription of viral genomic RNA into viral DNA by the viral reverse transcriptase; secondly, integration of viral DNA into host cell genome by viral integrase; and thirdly, cleavage of newly synthesized viral polypeptides by the viral protease into individual functional proteins for virion assembly and maturation. Following their discovery, all three viral enzymes have been considered targets for antiretroviral drugs.

Presently, there are several inhibitors against reverse transcriptase, integrase and protease that are playing a major role in the treatment and management of HIV-1 infection. All these inhibitors were synthesized based on HIV-1 subtype B genetic background and hence further research on other subtypes might give direction to the future discovery of more efficient and effective treatment. Since HIV-1 subtype C (HIV-1-C) overwhelmingly drives the epidemic in Southern Africa and accounts for more than 50% of infections worldwide, it is therefore an important variant in the study of the pathogenesis, treatment and prevention of HIV infections. Consequently, studies on the inhibition of HIV-1-C enzymes such as the protease, which exhibits high variability compared to subtype B protease, are required. In the current study, the protease of HIV-1-C was expressed and its activity evaluated.

The nucleotide sequence of HIV-1 subtype C protease gene was amplified from the plasmids MJ4 and IndieC by polymerase chain reaction, cloned into PHGB1 expression vector. Subsequently, BL21 DE3 cells were transformed with the clone and protein expression was then induced with isopropyl-β-D-thiogalactopyranoside (IPTG). The catalytic activity of the expressed enzyme was evaluated by incubating the enzyme with a synthetic HIV-1 peptide substrate containing HIV-1 protease cleavage sites. The high fluorescence reading obtained after incubation of the enzyme and the substrate

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indicated active proteolysis by the expressed enzyme. This study established a protocol to routinely express HIV-1 subtype C protease required for downstream studies such as the evaluation of molecules for anti-HIV-1 subtype C properties, and enzyme-substrate interactions. In addition, the established protocol can be adapted for the expression of other HIV-1 subtype C enzymes such as reverse transcriptase and integrase.

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