Kinetic and Thermodynamic Characterization of the South African Subtype C HIV-1 Protease: Implications for Drug Resistance

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DECLARATION

I declare that this thesis is my own, unaided work. It is being submitted for the degree of Doctor of Philosophy in the University of the Witwatersrand, Johannesburg. It has not been submitted for any degree or examination in any other University.

Salerwe Mosebi

this day of 2007

I dedicate this work to the precious people in my life who have unselfishly supported my choice of paths:

My parents: Abuti Thabo and Ausi Tshidi for all the endless support and encouragement over the years

My brother: Semana, you are the best brother in the world

In the memory of my grandparents: Papi and Mma Mosebi

"It is not hard to see that the possibility of scientific advance is closely connected with the role of heterodoxy, since new ideas and discoveries have to emerge initially as heterodox views, which differ from, and may be in conflict with, established understanding. The history of scientific contributions across the world – the experiences of Copernicus, or Galileo, or Newton, or Darwin – shows many examples of the part that resolute heterodoxy has to play, in scrutinizing, and when necessary rejecting, the views that are standardly accepted".

Professor Amartya Sen

ABSTRACT

The magnitude of the AIDS epidemic is well documented. It has been shown that Africa constitutes about 70 % of people infected with HIV worldwide. Efforts to control the AIDS epidemic have focused heavily on studies pertaining to the biology, biochemistry and structural biology of HIV and on the interactions between HIV proteins and new drugs. One of the most challenging problems in AIDS therapy is that HIV develops drug-resistant variants rapidly. Extensive research has been dedicated to designing resistance-evading drugs for HIV-1 protease (predominantly subtype B), which is crucial for the maturation of viral, structural and enzymatic proteins. There are 10 subtypes of HIV-1 within the major group of the virus, with subtype C accounting for about 95 % of infections in South Africa. Since HIV-1 antiretroviral treatment has been developed and tested against the B subtype, which is prevalent in North America, Western Europe and Australia, an important question relates to the effectiveness of these drugs against the C subtype. At this point, however, little is known about inhibitor-resistant mutations in the subtype C. The study, therefore, looked at the two active site mutations (V82A and V82F/I84V) in the South African HIV-1 subtype C protease (C-SA) emerging from the viral population circulating in patients. These mutations are well-characterized within the framework of the subtype B and are known to cause cross-resistance to most of inhibitors currently in clinical use. Protein engineering techniques were used to generate the V82A and the V82F/I84V variants. Comparative studies with the wild-type HIV-1 C-SA protease were performed. The spectral properties of the V82A and the V82F/I84V variants indicated no changes in the secondary structure in the respective variant proteins. Tryptophan and tyrosine fluorescence indicated a major difference in the intensities at the emission maxima for all three proteins. The fluorescence intensity of the V82F/I84V variant, in particular, was significantly enhanced indicating the occurrence of tertiary structural changes at/near the flap region. Both mutations did not impact significantly upon catalytic function. Both variants also had the same K_m values comparable to that of the wild-type enzyme. The catalytic efficiencies and the kinetic constants were lowered 3.6-fold for the V82A mutation and 6-fold for the V82F/I84V mutation relative to the wild-type C-SA protease. Inhibition studies were performed using four inhibitors in clinical use (saquinavir, ritonavir, indinavir and nelfinavir). For the V82A variant, IC_{50} and K_i values for saquinavir and nelfinavir

were not affected, whilst those for ritonavir and indinavir were 5- and 9-fold higher than the wild-type C-SA protease, respectively. Against the V82F/I84V variant, however, the inhibition constants were drastically weaker and characterized by IC_{50} and K_i ratios ranging from 50 to 450. Isothermal titration calorimetry (ITC) was also used to determine the binding energetics of saquinavir, ritonavir, indinavir and nelfinavir to the wild-type C-SA, V82A and V82F/I84V HIV-1 protease. The V82A mutation lowered the Gibbs energy of binding for the respective four clinical inhibitors by 0.4 kcal/mol, 1.3 kcal/mol, 1.5 kcal/mol and 0.6 kcal/mol, respectively, relative to the wild-type C-SA HIV-1 protease. The affinity of V82A HIV-1 protease for saquinavir, ritonavir, indinavir and nelfinavir (K_d = 1.85 nM, 2.00 nM, 12.70 nM and 0.66 nM, respectively, at 25 °C) was in the range of 2- to 13-fold of magnitude weaker than that of the wild-type C-SA protein. The clinical inhibitors exhibited the highest binding affinity to both the wild-type and the V82A enzymes, but were extremely sensitive to the V82F/I84V mutation. The V82F/I84V mutant reduced the binding of saquinavir, ritonavir, indinavir and nelfinavir 117-, 1095-, 474- and 367-fold, respectively. A drop in K_d values obtained for the V82F/I84V in association with saquinavir, ritonavir, indinavir and nelfinavir was consistent with a decrease of between 2.8 - 4.2 kcal/mol in ΔG, which is equivalent to at least 2 to 3 orders of magnitude in binding affinity. Taken together, thermodynamic data indicated that the V82A and V82F/I84V active site mutations in the C-SA subtype lower the affinity of the first-generation inhibitors by making the binding entropy less positive (unfavorable) and making the enthalpy change slightly less favorable.

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ABBREVIATIONS

ABC	abacavir
AIDS	aquired immune deficiency syndrome
ATV	atazanavir
AZT	zidovudine
bp	base pairs
CA	capsid protein
CD	circular dicroism
ΔCp	change in heat capacity
DMSO	dimethyl sulfoxide
DTT	1,4-dithiothreitol
DNA	deoxyribonucleic aicd
ddc	zalcitabine
ddI	didanosine
d4T	stavudine
3TC	lamivudine
DEAE	diethylaminoethyl
DLV	delavirdine
EDTA	ethylenediaminetetra-acetic-acid
EIAV	equine infectious anemia acid
EFV	efavirnez
fAPV	fosamprenavir
FDA	Food and Drug Administration
FTC	entricitabine
FIV	feline immunodeficiency virus
Gag	group specific antigen
ΔG	change in Gibbs free energy
ΔH	change in enthalpy
HIV	human immunodeficiency virus
HAART	highly active anti-retroviral treatment
IN	integrase
IDV	indinavir
IPTG	ß-isopropylthiogalactoside
ITC	isothermal titration calorimetry

K_a	association constant
K_d	dissociation constant
kDa	kilodalton
K_i	inhibition constant
K_m	Michaelis-Menten constant
MA	matrix protein
LPV	lopinavir
NFV	nelfinavir
NC	nucleocapsid protein
NNRTI	non-nucleoside reverse transcriptase inhibitor
NVP	nevirapine
ORF	open reading frame
PDB	protein data bank
PCR	polymerase chain reaction
PMSF	phenylmethylsulfonylfluoride
Pol	polymerase
PR	protease
RNA	ribonucleic acid
RNase	ribonuclease
rpm	revolutions per minute
RSV	Rous sarcoma virus
RT	reverse transcriptase
RTV	ritonavir
ΔS	change in entropy
SDS-PAGE	sodium dodecylsulfate polyacrylamide gel electrophoresis
SE-HPLC	size exclusion high performance liquid chromatography
SIV	simian immunodeficiency virus
SQV	saquinavir
TDF	tenofovir
TFA	trifluoroacetic acid
TF	transmembrane protein
THF	tetrahydrofuranylurethane
TPV	tipranavir
V82A	replacement of wild-type valine (V) with alanine at position 82

V82F/I84V	replacement of wild-type valine (V) with phenylalanine (F) at position 82 and of wild-type isoleucine (I) with valine (V) at position 84
V_m	maximum velocity

The IUPAC-IUBMB one and three letter codes for amino acids are used

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