The Study of Dynamic Properties and Interactions of the Complex of L76V Mutant and Wild-type HIV-1 Protease with Curcumin

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Abstract

Recently, the major problem in treating patients with HIV has been the discontinuous treatment for a long enough time. This behavior results in the development of resistant strains of the virus and increases the number of new HIV infections that could lead to a significant increase in levels of multidrug-resistant HIV. From previous studies, curcumin which is a substance in turmeric has been reported to inhibit HIV-1 Protease enzyme. To understand the dynamic properties and interaction between wild-type (WT) and L76V Mutation (MT) in HIV-1 Protease (HIV-1 PR) enzyme with the curcumin, we perform two classical molecular dynamics (MD) simulations of WT and MT of HIV-1 PR complexes with curcumin. From the results, the curcumin has stronger interactions with MT than WT HIV-1 PR as we found two hydrogen bonds in the mutant system. These hydrogen bonds are formed between the oxygen atom of curcumin and the oxygen and nitrogen atoms of amino acid in flap regions of HIV-1 Protease. Moreover, the structures of HIV-1 Protease and curcumin in the WT complex showed greater flexibility than that of MT complex. These data indicate that curcumin forms a more stable complex with the L76V mutation than the wild-type in HIV-1 Protease.

Keywords**:** *MD simulation, HIV-1 protease, Curcumin, L76V, Wild-type, Mutation*

1. Introduction

The human immunodeficiency virus type 1 protease (HIV-1 PR) is a virus-specific aspartic protease which is responsible for processing the polyproteins of gag and gag-pol during virion maturation and for the proliferation of the retrovirus. The activity of HIV-1 PR enzyme is essential for virus infectivity, thus it is one of the major targets for the treatment of the pandemic disease HIV/AIDS. HIV-1 PR is a homodimeric protein that consists of two identical polypeptides of 99 amino acids. Each monomer contains an extended β-sheet region (a glycine-rich loop) known as the flap. The flexible flap regions consisting of residues 48–50 and residue 50 lay at the tip of the HIV-1 PR flap. The flap region of both monomers constitutes in part of the substrate-binding site and plays an important role in substrate binding, and one of the two essential aspartyl residues, Asp25 and Asp25’ which lay on the bottom of the cavity (Brik, 2003). Recently, there have been many Food and Drug Administration (FDA)-approved protease inhibitors that are commercially available, including atazanavir (Reyataz), darunavir (Prezista), Fosamprenavir (Lexiva), and Saquinavir (Invirase).

However, HIV can become resistant to protease inhibitors. This means that the virus does not respond to medicine any longer. Drug resistance can happen when HIV changes, or mutates, in a person's body. Viruses do this to try and survive. When it mutates, it may become resistant to the medicines a person who is taking. This is a major impediment to protease inhibitor therapy. The development of inhibitor-resistant protease mutants due to the high mutation rate is caused by the absence of a proof-reading function in HIV reverse transcriptase (Condra, 1995). More than 100 mutations in the HIV-1 PR gene have been associated with drug resistance (Brik, 2003). Among these mutations, the valine substitution, instead of leucine, at position 76 in the HIV-1 protease (L76V) is considered a major mutation and seems to weigh most on Darunavir (DRV) susceptibility. DRV is second-generation inhibitors that were designed to target resistant variants of HIV-1 PR. Mutation L76V is associated with two- and six-fold decrease in susceptibility to lopinavir, darunavir, amprenavir, and indinavir. However, it is also linked with seven- to eight-fold increased susceptibility to first-generation inhibitor saquinavir and atazanavir (Young, 2010).

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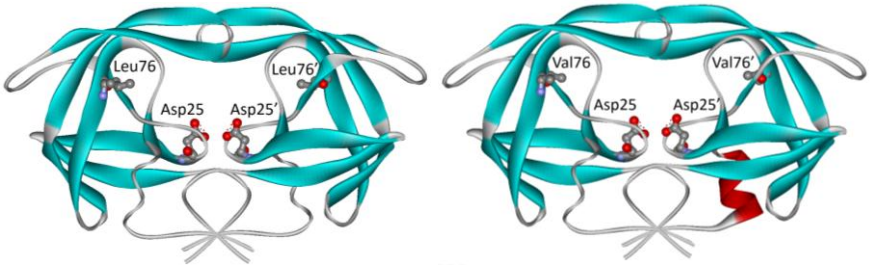

(a) (b)

Figure 1 Schematic representation of the crystal structure of WT (a) and MT (b) HIV-1 PR that use as starting structure

Curcumin is one of three curcuminoids present in turmeric (Zingiberaceae) or Curcuma longa Linn which is a medicinal plant widely cultivated in tropical regions of Asia. From previous research has demonstrated that curcumin is a potent anti-inflammatory agent that can reduce inflammation and may even play a role in cancer treatment. More recently, curcumin has been reported to inhibit HIV replication and was claimed for anti-HIV-1 and HIV-2 activities in a recent patent application (Pardee, 1994). In addition, curcumin and related compounds have also been reported for inhibition of HIV-1 and HIV-2 proteases with IC_{50} of 100 and 250 μM, respectively (Sui, 1993).

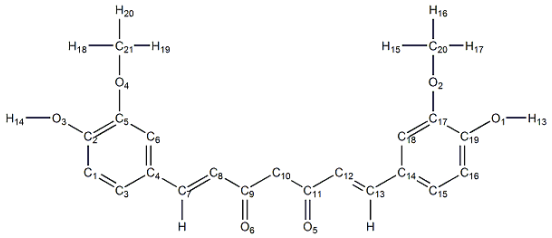

Figure 2 Molecular structure of curcumin

2. Objectives

The objectives of this research are to investigate the dynamic properties and interaction in molecular level of wild-type and L76V mutant HIV-1 protease as well as their binding with curcumin which is a substance in turmeric. Thus, the molecular dynamic (MD) simulations were carried out for the wild-type and L76V mutant HIV-1 protease complexes with curcumin in an explicit aqueous solution. This article reports about possible clinical advantages of a valine substitution, instead of leucine, at position 76 in the HIV-1 protease.

This mutation generally disappears quickly in replicating viruses in absence of selection pressure mediated by LPV, APV or DRV treatment. Thus, for deep salvage therapy situations in patients with strongly limited therapy options, it might be of advantage to maintain these drugs in treatment regimens to preserve L76V in the current replicating virus in combination with a "resensitized" drug ATV or SQ

3. Materials and Methods

3.1 Starting Structure

The 1.30 and 1.46 Å resolution crystallographic structures of the wild-type and L76V mutant HIV-1 PR complexes with darunavir were obtained from protein data bank (PDB) with PDB ID: 2IEN (Tie, 2004) and 3PWM (Louis, 2011), respectively. Both structures were used as the initial structure for the wild-type (WT) and single mutant (L76V) systems. All missing atoms and hydrogens of the enzyme were added using the LEaP module in the AMBER 9 package (Case, 2005). Darunavir in both crystal structures was removed, then, the curcumin was introduced into the enzyme pocket using a molecular docking method

using the AutoDock suite of programs. The system was solvated with a TIP3P water box (Jorgensen, 1983) and neutralized by the counterions. The water box extended 10 Å away from any solute atoms.

3.2 Molecular Dynamics Simulations.

Energy-minimization and MD simulations were performed using the SANDER module of AMBER 9.0 with the Cornell force field (Cornell, 1995). First, energy minimization was applied to water molecules using 3000 steps of the steepest descents and then 2000 steps of the conjugate gradients. Subsequently, the whole systems were subjected to energy minimization with 3000 steps of the steepest descents and 2000 steps of the conjugate gradients. The MD simulations were performed, employing the periodic boundary condition with the NPT ensemble. A Berendsen coupling time of 0.2 ps was used to maintain the temperature and pressure of the systems (Berendsen, 1984). The SHAKE algorithm (Ryckaert, 1997) was employed to constrain all bonds involving hydrogens. A simulation time step of 2 fs was used. All MD simulations were run with a 12 Å residue-based cutoff for nonbonded interactions, and the particle-mesh Ewald method was used for an adequate treatment of long-range electrostatic interactions (York, 1993). All MD simulations were gradually heated from 0 to 298 K in the first 60 ps. Then, the systems were equilibrated at 298 K for another 4000 ps. During heating, all solute atoms were restricted by a harmonic constraint with a strength of 10 kcal/mol.$Å^2$. Finally, 4000 ps production phase were performed without restriction at a constant temperature of 298 K and a constant pressure of 1 atm for all systems. The convergence of energies, temperatures, and pressures of the systems, and the atomic root-mean-square deviation (RMSD) of the enzyme and the inhibitor were used to verify the stability of the systems.

4. Results and Discussion

4.1 Structural Features of the HIV-1 Protease.

To explore the stability of MD simulations, the RMSD of backbone atoms of protein residues relative to the minimized structure was calculated (plotted in Figure 3). As observed from Figure 3, the RMSD values increased quickly in the first 1500 ps for WT and 500 ps for L76V, meaning that the structures of HIV-1 PR in solution relaxed and released repulsions within the complexes. In addition, the RMSD values of WT showed a slightly higher fluctuating than that of MT system. This data suggests that the WT system requires a longer time than L76V mutant to approach equilibrium and the HIV-1 PR in the MT system shows a little higher structural stability than the HIV-1 PR in the WT system.

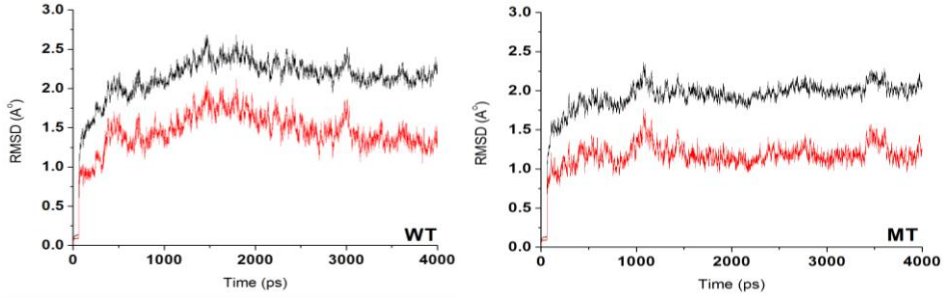

Figure 3 RMSD of backbone atoms of HIV-1 PR in all complexes relative to the starting structure

Figure 4 shows the superimposition of the final snapshot from the MD trajectories and starting structure of WT with L76V mutant. One can see that the global tertiary structures of the two systems are slightly different from the starting structure (Figure 4). A large difference can be seen on the active site and flap region of WT system. Considering the HIV-1 protease flaps, a greater change was found for the flaps in chain A which has a greater degree of the opening than that of chain B for WT. The degree of the flap change is simply demonstrated by a measure of the inter-residue distances of *d1* and *d2* as shown by the solid line in Figure 4.

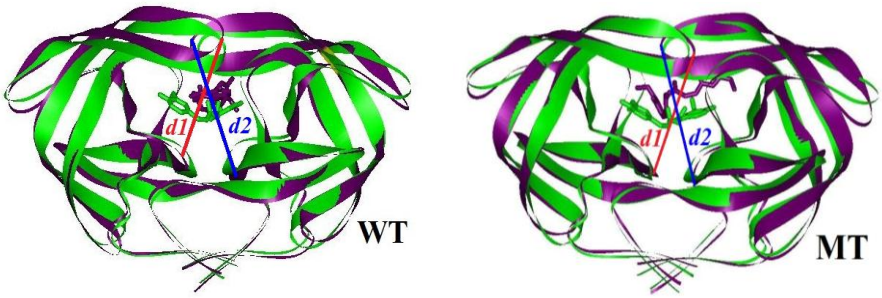

Figure 4 C_α trace of the starting structure (green) and the final snapshot from the MD (purple). Solid lines demonstrate the measured distances of C_α-Ile50/C_β-Asp25 (*d1*) and of C_α-Ile50'/C_β-Asp25' (*d2*)

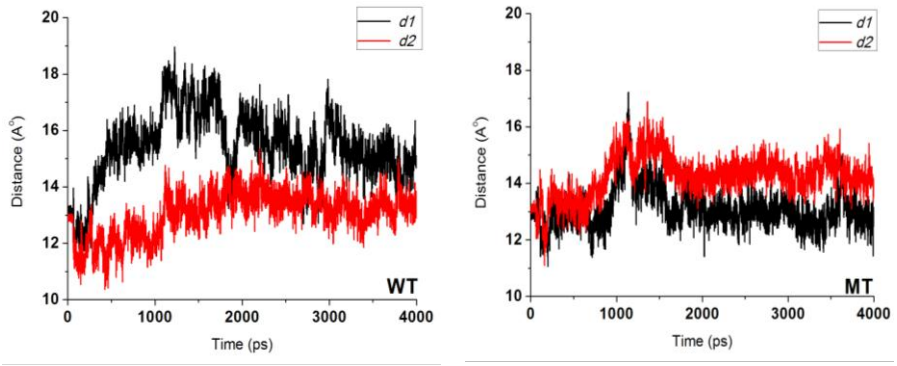

Figure 5 Distance trajectories of *d1* and *d2* (as shown in Figure 4)

From Figure 4 and 5, a slight shift of the inter-residue distance of chain B (*d2*) was observed for all systems, but a higher fluctuating of *d2* distance was found for L76V mutant. This implies that the flap opening was not found for chain B in both systems. The most shifted distance was found for a *d1* distance of WT. It is likely that the flap tip in chain A of the WT has a greater degree of the opening than that of L76V. This may relate to a loss of interactions of the curcumin in the binding pocket. This phenomenon might be involved to increase *d1* distance.

4.2 Hydrogen bonding

The hydrogen bonding was determined using the following criteria: a distance between donor and acceptor heavy atoms ≤ 3.5 Å and an angle of donor-H-acceptor ≥ 120°. From the results, no direct hydrogen bond was observed between curcumin and the catalytic residue in WT systems but we found two direct hydrogen bonds between curcumin and the flap residue in MT systems. It appears that for all complexes, the curcumin is flexible and move toward closing the flap region. It moves away from the catalytic residue and does not form hydrogen bonds with the catalytic residue as you can see from Figure 4. This behavior generated more free space in the active site, making it possible for a few water molecules to form hydrogen bonds with the active site. According to the hydrogen bond analysis, the oxygen atoms of curcumin was found to form two hydrogen bonds with the oxygen atom of Gly48 and the nitrogen atom of Gly49 of MT HIV-1 PR as shown in Figure 6. It should be noted that the mutation of L76V to a shorter side chain is expected to reduce the steric effect between protein and inhibitor and generated more free space in the binding site, leading to the conformational change and the hydrogen bonding of curcumin with flap residues in the mutant complex.

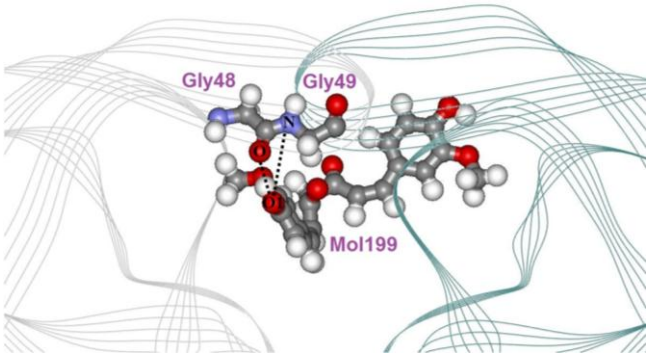

Figure 6 Hydrogen bonding between Gly48 and Gly49 of HIV-1 PR with curcumin

Table 1 Direct hydrogen bonding between curcumin and L76V HIV-1 PR

System	Donor (Protease)		Acceptor (Curcumin)		%Occupied
	Atom	Residue	Atom	Residue	
L76V	O	Gly48	O1	Curcumin	86.10
	N	Gly49	O1	Curcumin	52.40

5. Conclusion

MD simulations were performed to investigate the dynamic properties and interaction in molecular level of curcumin bound to wild-type and L76V mutant HIV-1 protease. From the results, we observed that the curcumin in both systems are flexible and move towards closing the flap region. This behavior generated more free space in the active site, making it possible for a few water molecules to form hydrogen bonds with the active site. Apparently, the mutation of L76V has a strong influence on the conformation of curcumin lead to different hydrogen bonding between HIV-1 PR and curcumin. The curcumin has stronger interactions with MT than WT HIV-1 PR because we find two hydrogen bonds in the mutant system. These hydrogen bonds are formed between the oxygen atom of curcumin with the oxygen and nitrogen atoms of amino acid in flap regions of HIV-1 Protease. In addition, the flaps of the wild-type HIV-1 PR open to a much greater degree than what was observed in mutant protease due to the loss of interactions between protease and curcumin. The detected data suggest us to conclude that curcumin was found to bind in different ways to the binding sites of both WT and L76V HIV-1 PR that is due to the mutation. These data indicate that curcumin forms a more stable complex with the L76V than the WT of HIV-1 Protease.

6. Acknowledgements

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7. References

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