Nano World Journal

https://doi.org/10.17756/nwj.2023-s5-047

Review Article

Open Access

HIV-1 Protease Drug-resistant Mutations and Inhibitor Binding Mechanism: A Review on Hybrid Quantum Mechanics/Molecular Mechanics Approach

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Received: October 20, 2023
Accepted: December 22, 2023
Published: December 28, 2023

Citation: Kumari N, Bamrah G, Biswas A, Srivastava S. 2023. HIV-1 Protease Drug-resistant Mutations and Inhibitor Binding Mechanism: A Review on Hybrid Quantum Mechanics/Molecular Mechanics Approach. *NanoWorld J* 9(S5): S240-S247.

Published by United Scientific Group

Abstract

HIV-1 (Human immunodeficiency virus type one) protease (PR) is a decisive enzyme for the replication of HIV-1. It is important in the progression of acquired immunodeficiency syndrome (AIDS). The viral enzyme integrase is required for HIV-1 replication because it permits viral DNA to be integrated into the DNA of the host cell. Because of this integration process, the virus is able to develop mutations in its protease, which leads to resistance to certain inhibitors. The current study aims to provide an overview of the molecular mechanisms underlying drug resistance in HIV-1 PR, which occurs as a result of various mutations. The computational studies involving hybrid quantum mechanics (QM) and molecular mechanics (MM) methods provides accurate data regarding the dynamics of the inhibitor-protease complex. This study contributes to a deeper understanding of the inhibitor-protease complex in various mutations and shed light on potential strategies to combat drug resistance in HIV-1 treatment.

Keywords

HIV-1 protease, Inhibitor, QM/MM simulation, Protein mutation

Introduction

The HIV, predominantly affects immune system cells called CD4 cells or T-helper cells, which takes on a pivotal role in maintaining a healthy immunological response. As the virus replicates within the body, it progressively weakens the immune complex, rendering the person susceptible to a range of infections and illnesses [1-3]. According to the World Health Organization's report from April 19, 2023, HIV continues to be a tough nut to crack for global public health community, causing the loss of around 40.1 million lives to date, with ongoing transmission observed in every country across the globe. Till the end of 2021 around 38 million people are infecting with HIV of which a major portion around 26 million are from Africa and around 2.1 million from India. Around 1.5 million people acquired HIV, and approximately to 650,000 people perished in 2021 as a result of HIV-related conditions [4]. The fight against HIV/AIDS remains a critical priority in global health efforts. HIV's life cycle depends on the HIV-1 protease's participation. The viral polyproteins are divided into smaller functional proteins that are necessary for viral replication, which is its main function. HIV-1 protease's structure and function have been studied using X-ray crystallography [4, 5]. It has been revealed that the PR is a homodimer made up of two identical subunits, each of which has 99 amino acids [6]. The maturation process mediated by the protease is fundamental the virus's ability to cause infection. Consequently, drug research now focuses on the HIV-1 PR, leading to the creation of different inhibitors aimed at inhibiting its protease activity [7].

HIV-1 protease (Hp) converts monomers A and B into a homodimer, as depicted in figure 1. Despite the complex C2 symmetry of the proteins, the separation of monomers A and B is made possible by the interaction of asymmetric peptide substrates and inhibitor molecules [8, 9] successfully.

HIV-1 PR is crucial for producing the structural proteins and viral enzymes needed for the development of fully-grown, disease-causing viral particles. Through membrane fusion, HIV infects T-cells throughout the replication process. Upon entering the cell, the viral RNA undergoes reverse transcription, transforming into DNA. Subsequently, the integrase makes it easier for this DNA to be absorbed into the host cell DNA. Serve as the host cells transcription and translation processes are used to synthesize the viral polyprotein [10]. The infected cell synthesizes polyproteins (HIV-1 Gag and Gag-Pol proteins) that act as the primary structural components responsible for virus assembly. The HIV protease plays a critical part in virus maturation by breaking down the polypeptides within these polyproteins, producing mature proteins. Hydrolysis of HIV protease Gag and Gag-Pol polyproteins are cleaved at multiple locations, resulting in the production of diverse structural proteins, including viral envelope glycoproteins and reverse transcriptase enzymes [4, 11]. The other resulting proteins due to cleavage include p2, p1, and p6, which are relatively small proteins with unknown activities. Additionally, p17 represents the capsid protein, p24 represents the matrix protein, and p7 represents the nucleocapsid protein. These proteins are vital for the assembly and structure of the virus [12]. Also, the integrase and protease are essential components of the newly formed virion particles (Figure 2). Each monomer's catalytic aspartic acid residues Asp25 and Asp25' aggregate at the dimer interface to form the enzyme's active catalytic region [13]. Above the active site, two flexible sheets abundant in glycine constitute the flap area. When the enzyme connects to a substrate, these flaps undergo a conformational change, sealing off the PR active site.

The native substrate of the enzyme adopts an elongated configuration and interacts with at least seven amino acid residues. These interactions are conventionally are referred to as P4 to P1 and P1' to P4' (Figure 3). Between the P1 and P1' residues, the amide link is broken. The remarkable specificity of the enzyme is due to each subsites affinity for specific kinds of side chains that fit its structure. While the S1 and S1' subsites display a predilection for hydrophobic residues, equivalent to the S3 and S3' subsites, despite the fact that the S2 and S2' subsites can tolerate both polar and hydrophobic side chains [15]. After locating the HIV-1 protease's active location, researchers formulated several inhibitors to impede its activity. The subsequent section will delve into the detailed mechanism of these various inhibitors with Hp.

Protease Inhibitors and Inhibition Mechanism

HIV-1 PR inhibitors belong to a category of antiretroviral drugs that have been specifically designed to target HIV-1 protease activity. These medications initially bind to active enzymes, interfering with their action and thereby

Figure 1: As retrieved from PDB, HIV-1 protease structure (https://www.rcsb.org/structure/4NKK), showing monomer A (Green) and B (Orange).

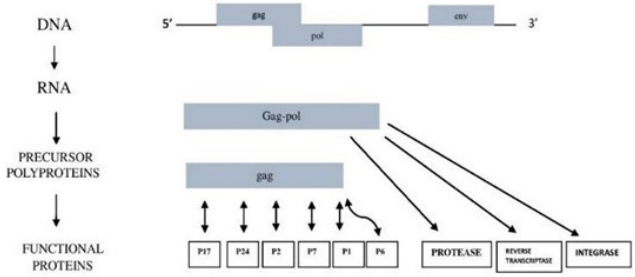

Figure 2: The HIV Gag-pol gene's translational products and the locations where the virus-encoded. Reproduced from [12].

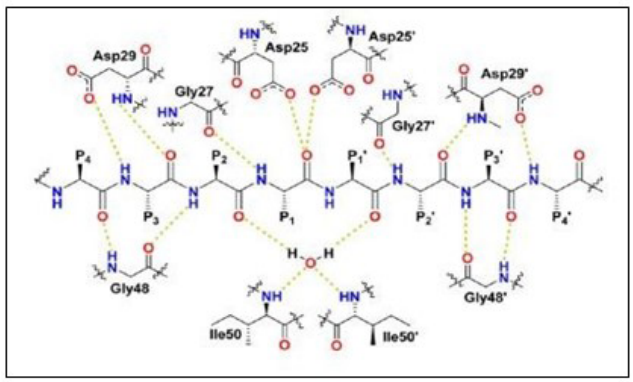

Figure 3: Hydrogen bonds in the active region of HIV protease, indicating the peptides' conserved binding mechanism taken from [14].

preventing their replication. By obstructing HIV-1 protease, these inhibitors effectively reduce the viral load in infected individuals, slow down the disease progression, and enhance immune function [10]. Figure 4 illustrates the structures of several approved inhibitors used for HIV treatment. Saquinavir (SQV), ritonavir, nelfinavir, amprenavir, and indinavir are first-generation inhibitors, whereas lopinavir, atazanavir, darunavir, and tipranavir are second-generation PR inhibitors [16].

The first-generation PR inhibitors had certain limitations. Many of these inhibitors were derived from peptides, resulting in limited oral absorption, short half-life, and high metabolic clearance. Consequently, frequent dosing was required to achieve adequate bioavailability. Additionally, constipation, diarrhea, and other abdominal discomfort were common gastrointestinal adverse effects of

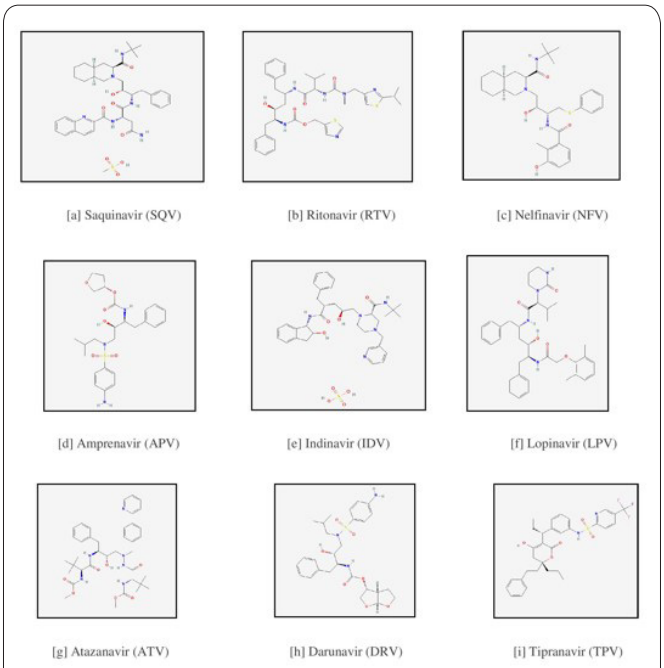

Figure 4: The structures of various HIV-1 protease inhibitors. The inhibitors **(a)** to **(e)** are the first generation whereas the inhibitors **(f)** to **(i)** are second generation inhibitors. The structures were taken from [14].

first-generation protease inhibitors. In addition, the advent of HIV strains that were resistant to these inhibitors posed a serious problem. To overcome these limitations, second-generation protease inhibitors incorporating hydroxyethylene and hydroxyethylamine isosteres were developed. Through their attachment to the catalytic aspartic acid residues, these isosteres mimicked the hydrolysis step's transition state [17].

To comprehend the mechanism of inhibitor binding, hybrid QM/MM methods were utilized. The hybrid QM/MM methodology is a computational approach that combines the accuracy of QM calculations with the efficiency of MM calculations. It is widely used in the field of computational chemistry to investigate complex systems where both the electronic structure and the molecular environment are crucial [18, 19]. SQV was the first wild type protease inhibitor to earn United States Food and Drud Administration approval in 1995. SQV binds to the protease's active site securely via non-polar van der Waals interactions enabled by its side chains, making tight contacts with both the flap and active site regions [20, 21]. Binding free energy calculated through the molecular dynamics (MD) simulations were found to be -186.39 KJ/mol, showing a strong binding with the protease [22]. The QM/MM reweighting free energy-self-consistent field method, a hybrid QM/MM free-energy technique, was used to conduct extensive research on another first-generation inhibitor, indinavir [23, 24]. Figure 5 and figure 6, indinavir is demonstrated to be present in the protease's active site, and the QM section of the computation is shown to investigate the binding mechanism. These approach employs iterative calculations and combines *ab initio* QM/MM geometry optimizations for the QM piece on a free-energy surface with long-term MD samplings for the MM component.

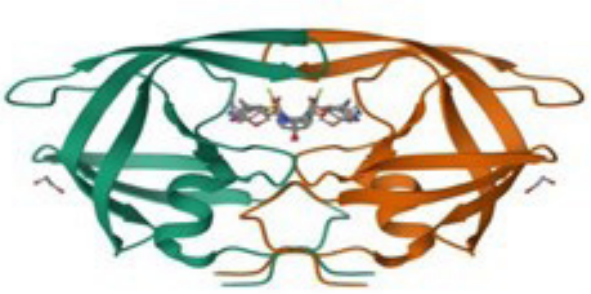

Figure 5: Diagram of an HIV-1 protease mutant coupled to indinavir as downloaded from PDB (https://www.rcsb.org/3d- view/2B7Z).

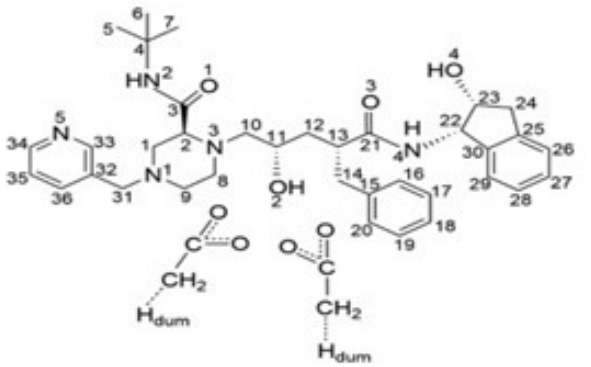

Figure 6: In the QM/MM RWFE-SCF calculations, the QM region of indinavir and the sidechains of Asp25(A) and Asp25(B) were used. Hdum dummy hydrogen atoms are also visible, capping the QM regions as taken from [30].

The optimization procedure continues until the MD statistical sampling and QM geometry optimization both converge simultaneously. This recursive process is known as sequential sampling [25-30]. By utilizing this method, researchers can obtain accurate and reliable results for the inhibitor-binding process, considering both quantum mechanical and molecular mechanical effects. The schematic diagram of QM region used by Taguchi et al. [30] is depicted in figure 5 showing aspartate side chains (Asp25(A), and Asp 25(B)) of monomer A and B included along with the indinavir molecule. It is as a result of indinavir's presence of an sp3 secondary alcohol structure near the catalytic Asp25 groups in the wildtype peptide substrate. Indinavir establishes a strong bond with the catalytic site of Hp because of its secondary alcohol structure, it resembles the diol form found in the transition state of the wild condition. This causes a potent inhibition of the enzyme's enzymatic activity [26-29]. Many additional inhibitors developed to date are also designed as transition state analogs, imitating the wildtype substrate of the enzyme's transition state. In both reactant and product phases, these inhibitors should bind to the enzyme more tightly than the substrate. Unfortunately, drug resistance has become a serious concern for most of the reported inhibitors due to mutations in HIV-1 PR. The next section will explore the mutations in the PR structure and the drug resistance caused by specific mutations.

Mutations and Drug Resistance in HIV-1 Protease

There are two kinds of HIV-1 PR mutations [31, 32]. Primary mutations occur in the active area of the enzyme and affect residues that are directly engaged in substrate binding. The protease's residues 25–32, 47–53, and 80–84 are thought to participate in the active site. On the other hand, secondary mutations affect residues other than those in the active site [33]. To reduce the unfavorable impact of primary mutations disrupting the interaction with the protease's native substrate, secondary mutations are frequently compensatory modifications that take place outside of the active region [14, 31]. Another class of secondary mutations involves changes to the Gag-Pol and Gag substrates' protein cleavage sites rather than the protease itself [34]. The inefficiencies brought on by primary mutations on protease activity are addressed by these alterations in cleavage sites, which are referred to as secondary mutations [35].

The direct contact between the protease and the protein inhibitor are typically altered by primary mutations, although rarely are residues involved in active catalysis [36]. These alterations, the propensity for protein-inhibitor binding is reduced yet proteolytic activity and beneficial interactions with the native substrate are kept. They are commonly found in the substrate binding sites [37]. Studies have demonstrated that the pocket where binding occurs position shifts to a disfavorable arrangement for protein inhibitors as a result of the active-site double mutation V82F/I84V. This shape modification results in fewer van der Waals interactions and significantly lowers binding enthalpy [36]. Secondary mutations are more common because the protease frequently needs further mutations to counteract the negative effects on substrate processing caused by primary mutations [38]. During protease inhibitor therapy, most of the first mutations have an impact on the active site of the enzyme, which directly affects inhibitor binding and is critical for protease inhibitor resistance [39]. Reports of resistance mutations that can be linked to protease inhibitors.

Table 1: List of HIV-1 protease mutations and the corresponding inhibitors for which they are resistant.

Inhibitor	Mutations involved
Saquinavir	48VM, 54VTALM, 82AT, 84V, 88S, and 90M
Indinavir	32I, 46IL, 47V, 54VTALM, 76V, 82ATFS, 84V, 88S, and 90M
Nelfinavir	30N, 33F, 46IL, 47V, 48VM, 54VTALM, 82ATFS, 84V, 88DS, and 90M
Amprenavir	V32I, G48V, I50V, I54V, and I84V
Fosamprenavir/ Ritonavir	32I, 33F, 46IL, 47VA, 50V, 54VTALM, 76V, 82ATFS, 84V, and 90M
Lopinavir	32I, 33F, 46IL, 47VA, 48VM, 50V, 54VTALM, 76V, 82ATFS, 84V, and 90M
Atazanavir	32I, 33F, 46IL, 47V, 48VM, 50L, 54VTALM, 82ATFS, 84V, 88S, and 90M
Darunavir	32I, 33F, 47VA, 50V, 54LM, 76V, 82F, and 84V
Tipranavir	32I, 33F, 46IL, 47VA, 54VAM, 82TL, and 84V

Table 1 lists the principal alterations and the individual inhibitors to which they are resistant [40].

It is crucial to understand that if the transition state (TS) analogue inhibitor accurately mirrored the wildtype substrate (WTS) during the TS, the mutations resulting in decreased binding affinity for an inhibitor would not occur [41-43]. If the inhibitor accurately replicated the chemical composition of the WTS in the TS, it would significantly reduce the mutant's enzymatic activity and eliminate the drug-resistant phenotype [44-52]. Therefore, simultaneous investigation of inhibitor binding and the enzyme the occurrence of the natural is necessary to design an inhibitor that is resistant to drug resistance. Asp25, the catalytic carboxyl group of the HIV-1 protease, has a particular protonation state that must be determined to comprehend and tailor inhibitor binding [49]. Their binding is strongly impacted by the fluctuation in the electrostatic environment at the protein-inhibitor interface. Because the hydrophobic groups of indinavir occupy the region around Asp25 in the binding form, the electrostatic environment around Asp25 in the inhibitor-binding state differs significantly from that in the protein without inhibitor binding [53].

Additionally, both the asymmetric peptide substrate and the inhibitor molecule shatter the protein's C2 symmetry when they bind to the homodimer Hp. The two carboxylic acids of Asp25 can have various protonation states based on how the substrate and inhibitor molecules are coupled, as mentioned above, even if the homodimer sequence of proteins exhibits symmetry. even if the homodimer the sequence of proteins exhibits symmetry. While the protonation states of proteins in the presence of different inhibitors have not been thoroughly investigated, Biochemical, nuclear magnetic resonance, and neutron diffraction techniques were used to investigate the protonation states of the original substrate and various inhibitors [54-60]. However, significant pharmacological resistance to HIV-1 PR inhibitors, such as indinavir, has developed and has been discovered [53, 61-72]. The binding affinity of indinavir is noticeably reduced by the V82T/I84V mutation, which raises the dissociation constant by a factor of 60-70. Surprisingly, an X-ray crystallographic experiment with a C-root-mean-square deviation of 0.2 showed no discernible structural similarities between the mutant and wildtype proteins [60]. Additionally, there are considerably more modest decreases in the mutant's enzymatic activity at some of the polyprotein's cleavage sites, leading to drug resistance [61].

A study was done to determine how the M46I mutation affected how HIV-1 PR interacted with the medicine SQV. The inhibitor primarily engages with the protein's flap region and active site through van der Waals and hydrogen bond interactions. The HIV-1 protease's-sheet structure, which is close to the flap region, underwent structural modifications because of the M46I mutation. Additionally, SQV binding changed the mutant protease's 50 helix. According to a MD's investigation, the mutation preserved the protease's open conformation upon inhibitor SQV binding while increasing motion in the flap area. The investigation discovered that the

mutation barely affected on the proteases active site. The mutant protease linked to SQV had more mobility than the wildtype protease, according to the examination of root mean square fluctuation, Asp25-Ile50/Ile149 distance, dynamic cross-correlation matrix, and principal component examination. A significant difference in binding energy was also seen between the SQV-M46I complex and the SQV-wildtype protease complex. Because the van der Waals energy contribution to the total binding energy had been significantly reduced, the altered protease-SQV complex showed less inhibitor binding. These findings show that the M46I mutation significantly affects the dynamics of the flap region of the mutant protease, which reduces its mutant protease's ability to bind with SQV [73].

Another evidence to support drug-resistance mechanism of the stated peptide inhibitors, Wang et al. [74] employed MD simulations to examine the conformational stability and local structural fluctuations brought on by the mutation G48T/L89M in HIV-1 PR when complexed with four inhibitors (APV, IDV, RTV, and NFV). According to the out-turn of MD simulations, when the mutant G48T/L89M residues were complexed with inhibitors, the changes in their side chains led to steric rearrangements of nearby residues, which had an impact on the pliability and associated movements of HIV-1 PR. Due to these structural modifications, the binding pocket's hydrogen bond occupancy decreased and the distances between the PR flaps and the catalytic sites significantly changed. The increased mobility of the flap region in the mutant complexes compared to the wildtype complexes impacted the interactions between inhibitors and key residues in the G48T/L89M mutant protease complexes. The reduction in van der Waals contacts between inhibitors and the mutant PR, as determined by energy calculations using MM Poisson-Boltzmann surface area and solvated interaction energy analyses, is the main cause of the G48T/L89M mutations' drug resistance. Additionally, the G48T/L89M mutations significantly changed the electrostatic energy and van der Waals energy of crucial residues near Ala28/Ala28' and Ile50/Ile50' with inhibitors in the mutant complex in compared to the wildtype complex, which primarily explains the drug resistance exhibited by these four mutations [74].

Meher et al. investigated the structural and dynamic characteristics of the TMC114 darunavir inhibitor in comparison to the HIV-1 protease double mutant I50L/A71V [75]. In particular, the tighter mobility of the flaps and the flip-flop contact between the catalytic Asp25 OD1/OD2 atoms and O18 of TMC114 were two features that stood out in their investigation as being in contrast. Energetic calculations further confirmed drug resistance by assessing the inhibitor's binding affinity. According to the structural energetic study, the drug resistance in the I50V mutant is predominantly caused by a decrease in the van der Waals energies of the residues Val50 and Val50' an increase in the polar solvation energy of Val50. Conversely, Leu50 and Leu50' have higher electrostatic and van der Waals energies than other flap residues Gly49', Ile84, and Ile47', which are all located in the active site, and this is primarily responsible for the higher binding affinity for the double mutant I50L/A71V. Like other protease inhibitors, the enhanced binding affinity of TMC114 suggests that it may be particularly suitable for the double mutant I50L/A71V. The article offers a comprehensive examination of the structure-affinity relationship, providing quantitative and mechanistic justifications for the mutational effect [75].

Conclusion

In conclusion, the development of AIDS and the replication of HIV-1 depend on the Hp. The viral enzyme integrase's activity is essential for the replication process because it enables the integration of a virus DNA copy into the host cells genome. This integration enables the virus to change its protease, resulting in resistance to particular inhibitors. This research study was to provide a comprehensive understanding of the MM underlying treatment resistance in Hp caused by various mutations. The electrical characteristics and dynamics of the inhibitor-protease complex have been precisely characterized by computational investigations using hybrid QM and MM approaches. These findings contribute to a deeper comprehension of the intricate interactions between inhibitors and protease, particularly in the presence of various mutations. Understanding the causes of the HIV-1 protease drug resistance will help develop new strategies to successfully tackle it and enhance the outcomes of HIV-1 treatment. Overall, this research emphasizes the crucial role of HIV-1 PR targeting in the fight against AIDS and provides insightful information on prospective ways to combat drug resistance. Ongoing research in this field offers hope for the development of more efficient medicines as well as an improvement in the standard of living for person with HIV-1.

Acknowledgements

Neha Kumari would like to thank Directorate of Research at Manipal University Jaipur for the Ramdas Pai Research Fellowship.

Conflict of Interest

None.

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