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Investigation of Darunavir resistance against triple mutant (V32I, I47V and V82I) in HIV-1 protease by molecular simulation approach

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Abstract: The HIV-1 Protease has been a critical drug target for war against AIDS and recently many protease inhibitors have been developed which inhibit the action of protease enzyme and prevent infection of HIV. However site-specific mutations occurring at one or more residues in HIV-1 protease has caused the development of resistance to protease inhibitors. In the present study, we have investigated the binding affinity of Darunavir to HIV-1 protease triple mutant (V32I, I47V and V82I) using bioinformatics approach. We have also highlighted the effect of mutations on the binding site residues and flap comprising residues in the HIV-1 protease by means of flexibility analysis. Molecular dockings were performed in order to gain insights into the binding mode of the Darunavir with HIV-1 protease structure. Subsequently, the docking results were also validated by means of PEARLS program. We hope that these results certainly will be helpful for the better understanding of mechanism of drug resistance.

Keywords: HIV-1 protease; Darunavir resistance; Normal mode analysis; Molecular docking; PEARLS.

Introduction

As well-known human immunodeficiency virus (HIV1) is one of the most dangerous virus for humans. The rapid spread of HIV-1 causing the acquired immune deficiency syndrome (AIDS) has evolved into a global problem with the infection number increasing in various ways. The pandemic diffusion of acquired immunodeficiency syndrome (AIDS) has promoted a series of efforts in order to understand and combat this lethal disease. There has been significant progress in AIDS treatment by highly active antiretroviral therapy (HAART) which combines three or more antiretroviral drugs.HIV type 1 protease is essential for the replication and assembly of virus whose activity is required for processing of Gag and Gag-pol polyprotein precursor into viral structure protein. Inhibition of the HIV-1 protease leads to the production of noninfectious virus particles.HIV PR has therefore become one of the major targets for anti-HIV treatment, and PR inhibitors (PIs) have proven to be highly effective antiretroviral drugs The HIV-1 protease consist of 99 residue is a homodimer. Saquinavir, Ritonavir, Darunavir, Indinavir, Nelfinavir, amprenavir, lopinavir, atazanavir, tipranavir are eight first generation drugs targeted HIV-1 protease.Darunavir (previously known asTMC114)[1, 2, 3, 4] is the second generation drug which is recently approved by the FDA. Darunavir is extremely potent against wild type HIV and shows a high genetic barrier to the development of antiretroviral resistance [5, 6]. It was suggested that major structural feature of the compound responsible for these favorable properties are a Pico molar binding affinity to the wild type PR binding site, the ability to form many backbone-to-backbone hydrogen bonds with the

PR substrate binding cleft and the ability to adopt a conformation that fit with in the "substrate envelop" of the active site [8, 9]. Invitro selection studies suggested that development of resistance against darunavir requires more individual mutation and develops more slowly than development of resistance to other PIs[10]. However in the course of treatment mutated form of HIV-1 PR is selected, which are resistant toward one or more protease inhibitor. HIV-1 protease able to evolve resistance by both active site and non-active site mutation. Some combination of active site and non-active site mutation can reduce the affinity of both inhibitor and substrate. Flap region play an important role in influencing both substrate and inhibitors binding. Other than flap region many researcher added water during simulating HIV-1 PR and found that water was another important factor for protease activity. In this work we carried out explicit solvent molecular dynamic (MD) simulation of wild type (WT) and mutant HIV-1 protease with darunavir drug. Darunavir can effectively suppress the wild type and mutant viral infectivity and replication.G48 located in the flap region is important for shaping the binding pocket of the active site. Mutation in the V32I, I47V, V82I leading to increase resistance after serial passage in the presence of darunavir drug. Mutation identified in the Gag-Pol [7] reading frame that influences the selection of darunavir -specific mutations. Darunavir have a high genetic barrier to resistance development, which is due to maintaining main chain hydrogen bonds by inhibitor flexibility. Darunavir designed to target drug resistant PR1 by introducing more hydrogen bond with main chain PR atom.

Materials and methods

Data set

The three-dimensional (3D) structures of native and mutant HIV-1 protease were taken from the crystal structures of the Brookhaven Protein Data Bank (PDB) [11]to carry out computational analysis. The corresponding PDB codes were 4DQB and 3S54 for the native and mutant type respectively. Both structures were solved with >2.0 Å resolution. Darunavir was used as the small molecule/inhibitor for our investigation. The SMILES strings were collected from PubChem, databases maintained in NCBI [12] and were submitted to CORINA for constructing the 3D structure of small molecule [13].

Identification of binding site residues

It was a challenging task to extrapolate a mechanism of action from the view of three dimensional structures. Detailed biochemical information about the enzyme can be used to design substrate or transition state analogues, which can then be bound into the enzyme for structure determination. These can reveal binding site locations and identify residues, which are likely to take part in the receptor–ligand interaction. From this, a catalytic mechanism can be proposed. In order to identify the binding residues in the structure of HIV-1 protease, we submitted the PDB ID: 3S54, a complex of mutant HIV-1 protease with Darunavir, into the PDBsum program.PDBsum provides summary information about each experimentally determined structural model in the Protein Data Bank (PDB). Some of its most recent features, including figures from the structure's key reference, citation data, Pfam domain diagrams, topology diagrams and protein-protein interactions. Furthermore, it now accepts users' own PDB format files and generates a private set of analyses for each uploaded structure.

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Computation of docking score between inhibitor and HIV-1 protease enzyme

We examined the receptor ligand binding efficiency by means of dock score. Dockingwas performed with help of Patch-Dock program[14].3D coordinates of native and triple mutant HIV-1 protease with the inhibitor Darunavir was submitted in PDB format with default parameters.Patch-Dock program relies on molecular shape representation, surface patch matching plusfiltering and scoring[15]. The Patch-Dock algorithm gives docking transformations with best molecular shape complementarity of ligand and protein. Patch-Dock algorithm divides the Connolly dot surface representation [16]of the molecules into concave, convex and flat patches. Then, complementary patches are matched in order to generate candidate transformations. Each candidate transformation is further evaluated by a scoring function that considers both geometric fit and atomic desolvation energy[17].Computational processing times of Patch-dock are increased by advanced data structures and spatial pattern detection techniques, such as geometric hashing and pose clustering.

Analysis of ligand-protein interaction energy

The quantitative understanding of molecular interactions that regulate the function and conformation of proteins can be facilitated by analysis of the energetics of small molecule ligand-protein interaction energy. We used a Web-based software called PEARLS (Program for Energetic Analysis of Ligand-Receptor Systems), for computing interaction energies of DRV- HIV-1 protease complex. PEARLS can be extensively used for ranking potential new ligands in virtual drug screening process. AMBER molecular force field, Morse potential and empirical energy functions were used to compute the ligand-receptor van der Waals, electrostatic, hydrogen bond and water mediated hydrogen bond energies [18]. A substantial degree of correlation between the computed free energy and experimental binding affinity was evident which suggests that PEARLS may be useful in facilitating energetic analysis of ligand-protein complex structure.

Flexibility of residues by Normal mode analysis

The mean square fluctuation of atoms relative to their average positions gives the quantitative measure of flexibilities of amino acids residues which can berelatedtothenormalized mean square displacements, $\langle R^2 \rangle$[19, 20]. Therefore $\langle R^2 \rangle$ analysis is likelytoprovidenewerinsights into protein dynamics, flexibility of amino acids and protein stability[21]. It is to be noted that protein flexibility is important for proteinfunction, rational drug design, and maintaining various types of interactions. The flexibility of amino acids in the drug binding pocket is considered significant with respect to the binding efficiency [22, 23]. In fact change in the binding site amino acid flexibility influences the binding free energies of protein-drug complex. Moreover, inhibitors and substrates bind in active site cavity between catalytic residues and flexible flaps comprising residues 45-55 and 45'-55' [24]. Hence we analyzed the normalized mean square displacements, $\langle R^2 \rangle$, of binding site residues and flap comprising residues of HIV-1 protease with the aidof Elnemo program[25] in orderto understand the flexibility of these residues both in the native and the mutant types of HIV-1 protease.

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Results and Discussions

Binding Residues Analysis

The binding site residues in the structure of HIV-1 protease were obtained from the PDBsum by using the complex structure. Both native and mutant structure was used for analysis. The PDBsum tool was used to illustrate the contacts between HIV-1 protease binding residues and Darunavir. It was interesting to note that, the residues such as ASP25, GLY27, ASP29, ASP30 makes hydrogen bond with darunavir and the other residue namely LYS23, GLY27, ALA28, VAL32, GLY48, GLY49, PRO81, VAL82, ILE84, ASP30, VAL47 makes hydrophobic interaction with the darunavir. Both native and mutant structure shows the total of 11 hydrogen bond interactions. Similarly the native and mutant structure shows the total of 13 and 16 hydrophobic interactions respectively.

Docking studies of HIV-1 protease enzyme with inhibitor

Proteins are the basis of the life process at the molecular level. The protein interaction is either with other protein or with small molecules. Many biological studies, both in academia and in industry, may benefit from credible high-accuracy interaction predictions. Here, we used Patch-Dock, a very efficient algorithm for protein-ligand docking for analysis. The PDB format of the two molecules and the receptor binding sites were uploaded in to the server. It was interesting to note that docking score of native structure is higher than the mutant structure. The result is shown in fig 1.

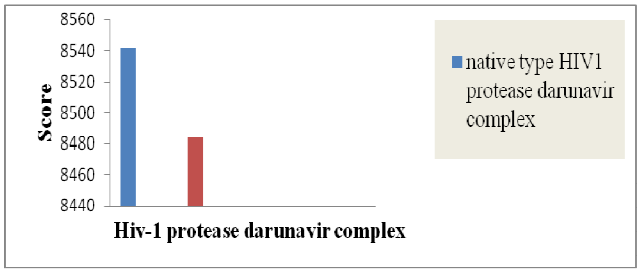
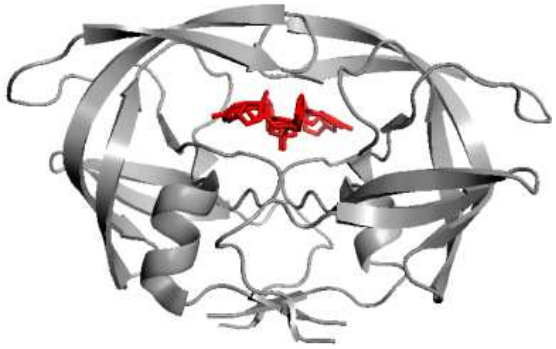


Fig 1: Plot between docking scores of native and mutant type HIV-1 protease complexes with darunavir

It is likely that the higher number of stabilizing residues makes the mutant (V32I, I47V and V82I) structure become highly stable and rigid. Hence Darunavir is not able to bind properly with the mutant structure. The complexes (native HIV-1 protease with Darunavir) and (triple mutant HIV-1 protease with Darunavir) are shown in Fig .2 (a) and (b) respectively which were obtained by using PyMOL software.

(a)



(b)

Fig 2: (a). Darunavir (red color) complex state with HIV-1 protease native type (4DQB) (**b).** Triple mutant (V32I, I47V and V82I) - complex state with HIV-1 protease mutant type (3S54)

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Ligand-receptor energetics analysis

The docking score obtained from the Patch-Dock program was further validated by means of PEARLS program. The result is shown in fig 3. We analyzed various energetic profiles of Ligand-Receptor Interactions by submitting the PDB format file corresponding to the native and mutant HIV-1 protease structures. The observations were made with respect to darunavir with the receptor HIV-1 protease. The total Ligand-Receptor Interaction Energy, Ligand-Receptor Van der Waals Energy, Ligand-Receptor Electrostatic Energy and Ligand-Receptor Solvation Free Energy were observed. It was interesting to observe that mutant HIV-1 protease-DRV complex showed lesser interaction energy then the native type HIV-1 protease. For instance, the total ligand-receptor interaction energy was found to be -15.08 kcal/mol for native complex structure whereas, it was found to be -10.49 kcal/mol for the mutant complex structure. This results correlate well with our docking score. Therefore, we confirm that mutations (V32I, I47V and V82I) in the HIV-1 protease structure significantly alter the binding of DRV.

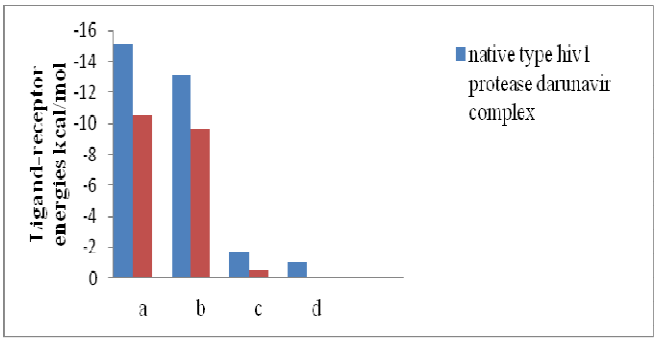

Fig 3: Plots showing comparisons between various Ligand receptor interaction energies of native and Mutant type HIV protease-Darunavir complexes. **a** total ligand-receptor interaction energy. **b** ligand receptor van der Waal's energy. **c** ligand-receptor electrostatic energy. **d** ligand receptor solvation free energy.

Flexibilities of binding site residues by Normal mode analysis

The binding site residues in the structure of HIV-1 Protease wereobtained from the LCT program using the complex structure of HIV protease-Darunavir (PDB ID: 3S54). The result is shown in fig .4 which indicates that a total of 30 amino acid residues act as binding residues in HIV-1 protease.

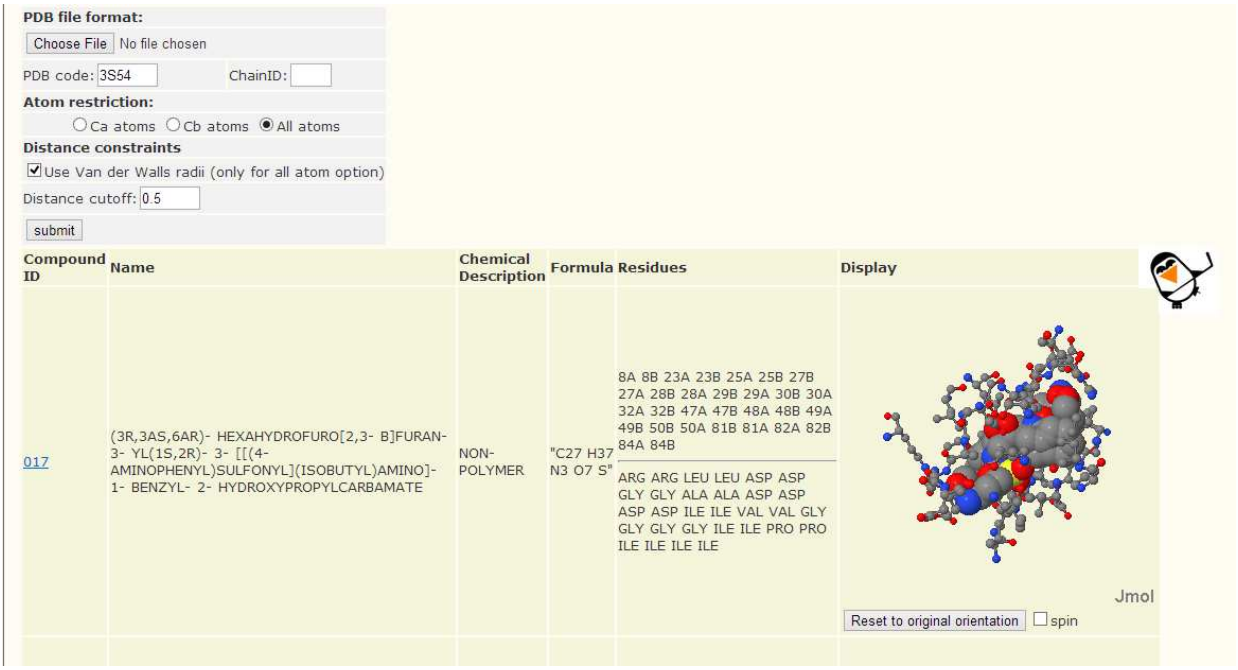

Fig 4: Ligand Contact tool showing binding site residues in the HIV-1 protease structure

Binding residue flexibility was fundamental to understanding the ways in which drug exerts biological effects. This flexibility allows decreased affinity to be achieved between a drug and its target enzyme. In order to understand the cause of drug insensitivity by mutations V32I, I47V and V82I, we used the program ElNemo [25] to compare the flexibility of amino acids of both native and mutants, which are involved in binding with Darunavir. Table 1 depicts the flexibility of amino acids in the drug-binding pocket of both native and mutants by means of normalized mean square displacement, <R2>. We further sorted out these data into three different ranges of flexibility. One is the<R2>of amino acids in the drug-binding pocket of mutants which is exactly the same as<R2>of the amino acids in the drug-binding pocket of natives named as "identical flexibility." The second was the<R2>of amino acids in the drug-binding pocket of mutants which is higher than<R2> of the amino acids in the drug-binding pocket of natives named as "increased flexibility" And the last is the<R2>of amino acids in the drug-binding pocket of mutants which is lesser than<R2>of amino acids in the drug-binding pocket of native named as "decreased flexibility." From the above classification, we understand that 80 % of drug-binding amino acids were in the range of decreased flexibility and 13.33 % of drug binding amino acids were in the range of increased flexibility (Table 1).This evidently exemplified that majority of amino acids participated in the drug-binding pocket of these mutants had flexibility decreased due to their occurrence in the range of "decreased flexibility" which signifies the loss of binding efficiency with the inhibitor, Darunavir.

Table 1: Comparison of Normalized mean square displacement of drug binding amino acids of native and mutant HIV-1 protease

S. No.	Binding residues	Normalized mean square displacement, $<R^2>$ in native type	Normalized mean square displacement, $<R^2>$ in Mutant type
1.	ARG8(A)	0.0139	0.0127[a]
2.	ARG8(B)	0.0127	0.0124[a]
3.	LEU23(A)	0.0135	0.0135
4.	LEU23(B)	0.0145	0.0137[a]
5.	ASP25(A)	0.0138[b]	0.0146
6.	ASP25(B)	0.0160	0.0151[a]
7.	GLY27(A)	0.0156	0.0152[a]
8.	GLY27(B)	0.0156	0.0151[a]
9.	ALA28(A)	0.0152	0.0144[a]
10.	ALA28(B)	0.0147	0.0147
11.	ASP29(A)	0.0150	0.0133[a]
12.	ASP29(B)	0.0131[b]	0.0136
13.	ASP30(A)	0.0151	0.0128[a]
14.	ASP30(B)	0.0123[b]	0.0124
15.	ILE32(A)	0.0081	0.0053[a]
16.	ILE32(B)	0.0049[b]	0.0050
17.	VAL47(A)	0.0360	0.0290[a]
18.	VAL47(B)	0.0396	0.0280[a]
19.	GLY48(A)	0.0410	0.0352[a]
20.	GLY48(B)	0.0375	0.0353[a]
21.	GLY49(A)	0.0412	0.0368[a]
22.	GLY49(B)	0.0409	0.0363[a]
23.	ILE50(A)	0.0353	0.0342[a]
24.	ILE50(B)	0.0397	0.0339[a]
25.	PRO81(A)	0.0318	0.0300[a]
26.	PRO81(B)	0.0305	0.0291[a]
27.	ILE82(A)	0.0260	0.0243[a]
28.	ILE82(B)	0.0236	0.0233[a]
29.	ILE84(A)	0.0120	0.0118[a]
30.	ILE84(B)	0.0126	0.0120[a]

[a]Amino acids with decreased flexibility of mutant than wild structure
[b]Amino acids with increased flexibility of mutant than wild structure

Flexibilities of Flap comprising residues

The available evidences suggest that substrate bind in the active site cavity between the catalytic residues and the flexible flaps comprising residues 45-55 and 45`-55` [24]. Moreover Flap region of protease secures the substrate within binding cleft with its opening and closing to hold substrate rigidly. Hence, analyzing the flexibility of flap region could be certainly helpful for the molecular level understanding of DRV resistance in the HIV-1 protease triple mutant. Val32 forms hydrophobic contacts with Ile47 in open conformation which lies in the Flap region [26]. Table 2 shows the $<R^2>$ values of flap residues of native and mutant HIV-1 protease obtained by low frequency normal mode of Elnemo program. It is interesting to observe in Table 2 that all the flap comprising residues in the mutant structure had flexibilities different than the native structure. For instance 81.81% decreased flexibility was observed in the flap region.

Table 2: Comparison of Normalized mean square displacement of residues comprising flexible flaps of native and mutant HIV-1 protease

S. No.	Binding residues	Normalized mean square displacement, $<R^2>$ in native type	Normalized mean square displacement, $<R^2>$ in Mutant type
1.	LEU45	0.0307	0.0263[a]
2.	MET46	0.0363	0.0289[a]
3.	ILE 47	0.0360	0.0290[a]
4.	GLY 48	0.0400	0.0352[a]
5.	GLY 49	0.0412	0.0368[a]
6.	ILE 50	0.0353	0.0342[a]
7.	GLY 51	0.0423	0.0411[a]
8.	GLY 52	0.0490	0.0441[a]
9.	PHE53	0.0461	0.0381[a]
10.	ILE 54	0.0370	0.0282[a]
11.	LYS 55	0.0284	0.0185[a]
12.	LYS 45	0.0332	0.0257[a]
13.	MET 46	0.0274	0.0283
14.	ILE 47	0.0296	0.0280[a]
15.	GLY 48	0.0375	0.0353[a]
16.	GLY 49	0.0409	0.0363[a]
17.	ILE 50	0.0397	0.0339[a]
18.	GLY 51	0.0469	0.0404[a]
19.	GLY 52	0.0477	0.0445[a]
20.	PHE 53	0.0370	0.0384
21.	ILE 54	0.0273	0.0277
22.	LYS 55	0.0100	0.0168

[a]Amino acids with decreased flexibility of mutant than wild structure

This certainly indicates their more involvement in H-bonding with the partner molecule. Thus, makes the complex structure unstable. Hence, we confirm that DRV resistance in the triple mutant is mainly because of the altered flexibility of binding site residues and flap region which is relevant to the experimental findings [27].

Conclusions

In the present study, we analyzed the impact of triple mutant (V32I, I47V and V82I) in the binding of DRV by molecular docking and normal mode analysis. We hope that these results support drug discovery projects forgenerating novel pharmaceutically active agents with desired properties and different bindingpatterns in a cost- and time-efficient manner.

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