Comparative study of some energetic and steric parameters of the wild type and mutants HIV-1 protease: a way to explain the viral resistance

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Abstract

Because, *in vivo*, the HIV-1 PR (HIV-1 protease) present a high mutation rate we performed a comparative study of the energetic behaviors of the wild type HIV-1 PR and four type of mutants: Val82/Asn; Val82/Asp; Gln7/Lys, Leu33/Ile, Leu63/Ile; Ala71/Thr, Val82/Ala. We suggest that the energetic fluctuation (electrostatic, van der Waals and torsion energy) of the mutants and the solvent accessible surface (SAS) values can be useful to explain the viral resistance process developed by HIV-1 PR. The number and localization of enzyme mutations induce important modifications of the van der Waals and torsional energy, while the electrostatic energy has an insignificant fluctuation. We showed that the viral resistance can be explored if the solvent accessible surfaces of the active site for the mutant structures are calculated. In this paper we have obtained the solvent accessible surface for a group of 15 mutants (11 mutants obtained by Protein Data Bank (PDB) file, 4 mutants modeled by CHARMM software) and for the wild type HIV-1 PR). Our study try to show that the number and localization of the mutations are factors which induce the HIV-1 PR viral resistance. The larger solvent accessible surface could be recorded for the point mutant Val 82/Phe.

Keywords: genomics • genetic code • genomic signals • complex representation • phase analysis • unwrapped phase • sequence path

Introduction

The Human Immunodefficiency Virus type 1 protease (HIV-1 PR) plays a critical role in the viral replication process by its involvement in the hydrolysis of the viral protein precursors [1, 2]. HIV-1 PR, a homodimer of known crystal structure with 99 aminoacids per chain, is included in the eukariotic aspartic protease family [3-5]. The β-sheet configurations, which include the triplet active site Asp 25/125-Thr 26/126-Gly 27/127 are present in the major part of the enzyme (aminoacids 1-85/101-185), whereas the α-helix domain covers the aminoacids 86-99 [3-6] (Fig. 1).

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It was proved by mutagenesis [7] that the aspartic acids (25, 125) are principally involved in maintenance of the active state of the enzyme, more than the residues Thr (26, 126) - Gly (27, 127) [8, 9]. Kinetic and computational studies [10-12] show that the viral protease presents a high mutagenesis rate, thus it is able to develop strong resistance to inhibitors [10-13]. Furthermore, computational techniques as molecular energy minimization [14], molecular docking techniques [6, 15], molecular dynamics simulations [16-19] or QSAR procedures [20-25] can be useful tools for the study of the HIV-1 PR mutants and their inhibitors. In the last years, the molecular volume and solvent accessible surface (SAS) [26] present a real interest for HIV-1 PR inhibitors design [6]. Also, the QSAR study used the molecular volume and solvent accessible surface [24] for obtaining a correlation between the predictable and experimentally measured biological activity of the HIV-1 PR inhibitors.

The aim of this paper is to offer a plausible explanation of the mutant HIV-1 PR resistance, obtained by calculating the solvent accessible surface [27] of the viral protease cavity, which can be considered to depend on: (i) the number of mutated aminoacids (in our study we included HIV-1 PR structures which have one or more mutations); (ii) the location of mutation sites (closely or away from the active site).

We computed the solvent accessible surface, using the – TINKER 3.9 software (Software Tools for Molecular Design develops by Jay Ponder Lab., Dept. of Biochemistry & Molecular Biophysics, Washington University School of Medicine, St. Louis, Missouri 63110, http://dasher.wustl.edu/tinker/, free for academic use) [28, 29] and ICMLite software (Software for Molecular Graphics and Data Analysis, Plotting and Statistics, develops by Molsoft, L.L.C., P.O. Box 113, Metuchen, NJ 08840, http://sal.kachinatech.com/Z/2/ICMLITE.html, free for academic use) [30, 31] for 15 HIV-1 PR mutants. We found that the HIV-1 PR mutants have very different SAS values. In our opinion, a comparative analysis of the solvent accessible
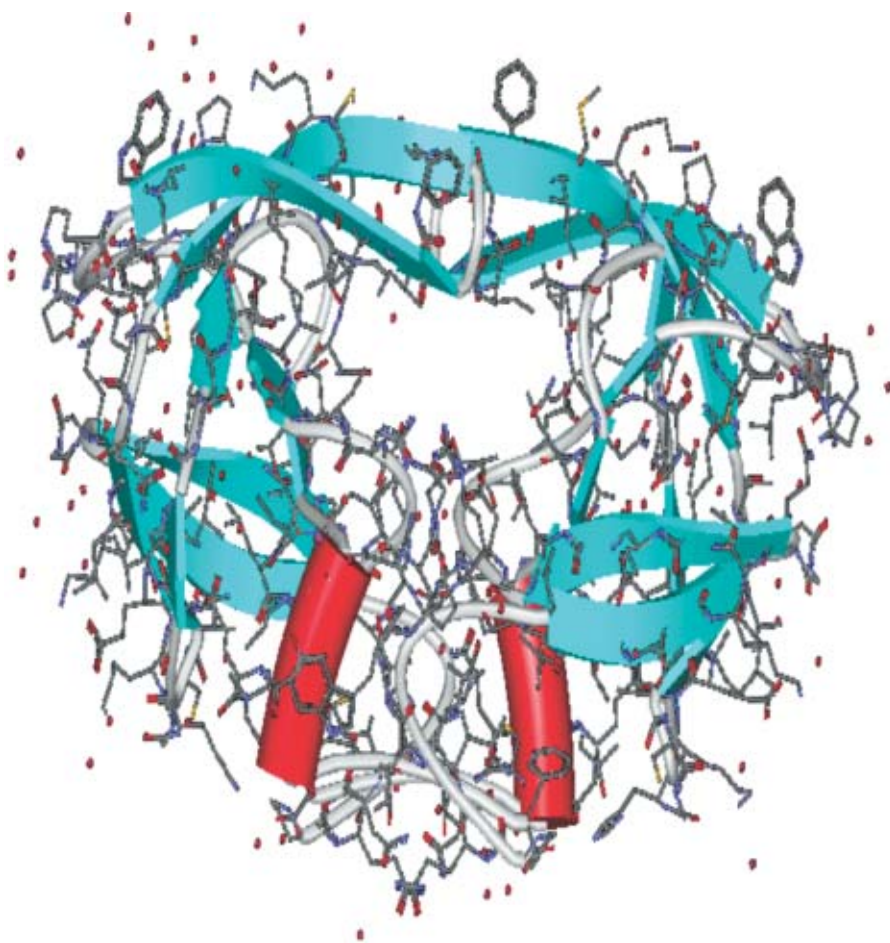

Fig. 1 The spatial distribution of HIV-1 PR secondary structure. arrow-β-sheet (aminoacids 1-85/ 101-185), tube - α helix. (aminoacids 86-99/186-198). - worked in the WebLab Viewer software, Molecular Simulations Inc., (MSI), http://msi.com, version demo - free for academic use-

Table 1. The number and positions of mutations in the mutant HIV-1 PR; c-mutations localized in close position to the active site. a-mutations localized away from the active site.

Number of mutations	Type of mutation and spatial localization of the mutations relative to the active site
Single	a-Cys95/Ala
	c- Val82/Asn
	c-Val82/Ala
	c-Val82/Asp
	c-Val82/Phe
	c-Gly48/His
	c- Ile84/Val
Double	a-Ala71/Thr, c- Val82/Ala
	c-Val82/Phe, c-Ile84/Val
	c-Val82/Phe, a-Cy95/Ala
	c- Ile84/Val, a-Cys95/Ala
Triple	a-Leu33/Ile, a-Cys67/ABA,a-Cys95/ABA
	a-Gln7/Leu, a-Leu33/Ile, a-Leu63/Ile
Multiple	a-Thr31/Ser, a-Ile32/Val,a-L33/Val, a-Glu34/Ala, a-Glu35/Gly, a -Met36/Ile, a-Ser37/Glu
	a-Thr31/Ser, a-Ile32/Val, a-L33/Val, a-Glu34/Ala, a-Glu35/Gly, a-Met36/Ile, a-Ser37/Glu, c-Ile47/Val, c-Val82/Ile

surface of the viral protease cavity can be very useful to evaluate the steric compatibility of the HIV-1PR active site with its inhibitors.

Materials and methods

Molecular modeling

We have chosen the mutant HIV-1 PR structures following certain criteria: (i) the mutants structures have from single to nine mutations; (ii) the HIV-1 PR mutations are localized closely (e.g. Val82/Asp and Ile84/Val) or far away from the active site (e.g. Leu 33/Ile,Cys67/ABA,Cys95/ABA).

The mutants: Val82/Asn;Val82/Asp; Ala71/Thr, Val82/Ala; Gln7/Lys, Leu33/Ile, Leu63/Ile were analyzed by CHARMM software (Chemistry at HARvard Macromolecular Mechanics develops by Professor Martin Karplus, Department of Chemistry & Chemical Biology, Harvard University, 12 Oxford Street, Cambridge, MA 02138) [32]. The HIV-1 PR mutants structures were obtained starting from the wild type (http://www.rcsb.org/pdb/, Brookhaven National Laboratory, Protein Data Bank, file 1QBS) [33].

Minimum energy

Minimization steps of the HIV-1 PR mutants and wild type were run by CHARMM software following the conditions: (i) the algorithm of minimization-Adopted Basis Newton Raphson; (ii) the number of minimization steps was 25000; (iii) the gradient tolerance = 0.00001; (iv) the cut-off radius = 11.0 Å; (v) the solvent environment contained 200 molecules of crystallized water. During the minimization process we recorded the electrostatic, van der Waals and torsional energy in accord with the classical Coulomb, Lennard-Jones 6-12 and torsion potential.

Calculation of the solvent accessible surface

Computing the solvent accessible surface for the HIV-1 protease-wild type and mutants could gives us the possibility to realize a comparative study of the steric interactions among HIV-1 PR mutants and their inhibitors. The number and localization of mutations are given in Table 1.

Starting from the hypothesis that the molecules which are characterized by a similar solvent accessible surface have similar biological features, we suggested that the solvent accessible surface of the HIV-1 PR active site can be useful to explain the viral inhibition mechanism. For a better interpretations of the enzymatic
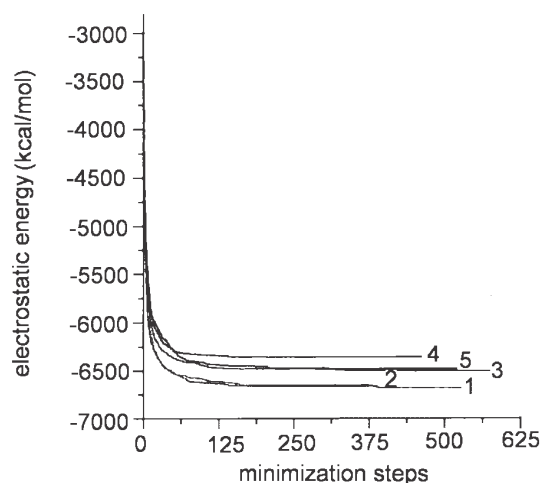
Fig. 2a The electrostatic potential of HIV-1 PR wild-type and mutants collected during the minimization process:1. wild-type(-6683.8kcal/mol); 2. Ala 71/Thr, Val 82/Ala(-6663.9 kcal/mol); 3. Gln7/Lys, Leu 33/Ile, Leu63/ Ile(-6484.0 kcal/mol); 4.Val 82/Asn(-6358.4 kcal/mol); 5. Val82/Asp(-6505.8 kcal/mol); -worked in CHARMM software-
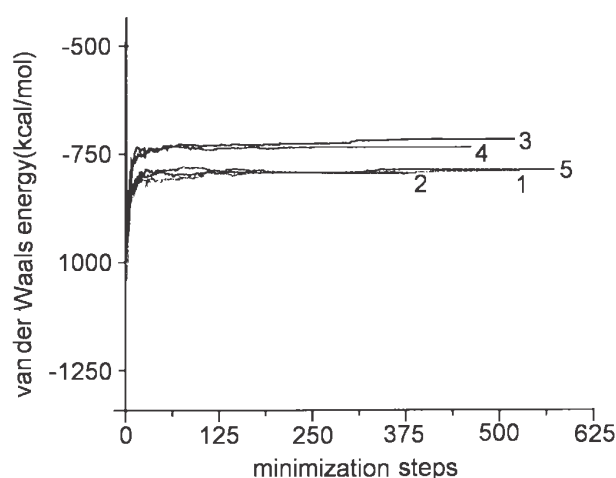

Fig. 2b The van der Waals energy of HIV-1 PR wild-type and mutants collected during the minimization process 1. wild-type(-791.0 kcal/mol); 2. Ala71/Thr, Val82/ Ala(-796.3 kcal/mol); 3. Gln7/Lys, Leu33/Ile, Leu63/Ile(-717.8 kcal/mol); 4. Val 82/Asn(-735.7 kcal/mol); 5. Val82/Asp(-788.2 kcal/mol) - worked in CHARMM software-
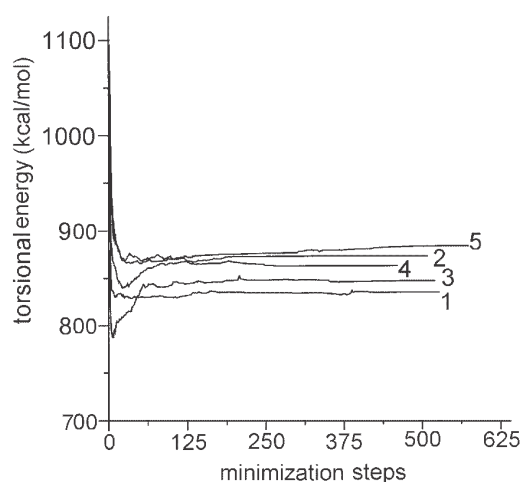

Fig. 2c The torsion energy of HIV-1 PR wild-type and mutant collected during the minimization process : 1. wild-type(833.7 kcal/mol); 2. Ala71/Thr, Val82/Ala(847.4 kcal/mol); 3. Gln7/Lys, Leu33/Ile, Leu63/Ile(782.5 kcal/mol); 4. Val82/Asn(861.9 kcal/mol); 5. Val82/Asp(882.3 kcal/mol). - worked in CHARMM software-

reactions, we calculated the solvent accessible surface for residues: 29, 30, 47, 48, 49, 50, 80, 82, 84 belonging to the A chain (first monomer of HIV-1 PR) and the homologue residue 29', 30', 47', 48', 49', 50', 80', 82', 84' belonging to the B chain (second monomer of HIV-1 PR). These residues are present in the proximity of the active site. For more accurate results, we used TINKER 3.9 and ICMLite software. We obtained a good agreement between the solvent accessible surface calculated by TINKER 3.9 and ICMLite software

Results

Electrostatic force fields of mutant structures

By comparing the electrostatic energy of the HIV-1 PR mutants and wild type we noticed that: (i) the electrostatic energies are very close, especially for Ala71/Thr,Val82/Ala mutant (-6663.9 Kcal/mol) and the wild-type (-6683.8 Kcal/mol); (ii) the same observation can exist for the Val82/Asn mutant (-6358.4 Kcal/mol), the Val82/Asp mutant (-6505.8 Kcal/mol) and the Gln7/Lys, Leu33/Ile,

Leu63/Ile mutant (-6484.0 Kcal/mol) (Fig. 2a). Therefore, we suppose that, during the minimization process, the intramolecular electrostatic interactions in the HIV-1 protease mutants and wild type are not essentially modified.

Effect of mutations
on the van der Waals energy

The change of van der Waals energy induced in the Val82/Asp (–788.2 Kcal/mol), Ala71/Thr,Val82/Ala (-796.3 Kcal/mol) mutants compared to the wild type HIV-1 PR (-791.0 Kcal/mol) is insignificant. This fact suggests that the presence or absence of methyl groups from the Val82 residue, do not induce a clear modification of the van der Waals

effect. For the Gln7/Lys, Leu33/Ile, Leu63/Ile mutant, the van der Waals effect produced by the presence of four methylen groups, from the Lys residue, gives a van der Waals energy of -717.8 Kcal/mol (Fig. 2b). Different values of the van der Waals energy recorded for the point mutants Val82/Asn (-735.7 Kcal/mol) and Val82/Asp (–788.2 Kcal/mol) are noticed. Unfortunately, we have not a clear explanation about the major difference of the van der Waals energy for the mutants Val82/Asn and Val82/Asp, taking into account that both mutants lose the methyl groups from Val82 and gain the hydrophilic groups. But, we suppose that the presence or absence of one or more hydrophobic groups in this position (Val82) is not sufficient to explain the van der Waals force field induced in the HIV-1 protease mutants.

Table 2. The solvent accessible surface values for the HIV-1 active protese cavity-wild type and mutants using ICMLite and TINKER 3.9 software.

Type of mutation	PDB code	SAS (Å^2)a	SAS (Å^2)b
Gly48/His	1a9m	2393.6	2353.5
Thr31/Ser, Ile32/Val, L33/Val, Glu34/Ala,Glu35/Gly, Met36/Ile, Ser37/Glu	1bdl	2367.4	2324.8
Thr31/Ser, Ile32/Val, L33/Val, Glu34/Ala, Glu35/Gly, Met36/Ile, Ser37/Glu, Ile47/Val, Val82/Ile	1bdq	2387.9	2274.3
Val82/Phe, Cys95/Ala	1bv7	2441.1	2443.5
Ile84/Val, Cys95/Ala	1bv9	2336.3	2391.9
Val82/Asp	modeled in CHARMM	2196.1	2127.5
Gln7/Leu, Leu33/Ile, Leu63/Ile	modeled in CHARMM	2343.1	2348.0
Val82/Ala	1hvs	2307.8	2288.6
Ile84/Val	1mes	2310.7	2292.3
Val82/Phe, Ile84/Val	1meu	2470.3	2466.0
Val82/Phe	1met	2478.0	2477.0
Leu33/Ile, Cys67/ABA, Cys95/ABA	1mtr	2285.6	2305.1
Ala71/Thr, Val82/Ala	modeled in CHARMM	2336.0	2301.1
Cys95/Ala	1bvg	2450.8	2463.6
Val82/Asn	modeled in CHARMM	2307.6	2302.8
wild type	1qbs	2410.2	2380.2

a solvent accessible surface obtains by ICMLite software,
b solvent accessible surface obtains by TINKER 3.9 software.

Torsional energy variation for enzyme structures

We noticed that the wild type has a torsional energy of 833.7 kcal/mol. The Gln7/Lys, Leu33/Ile, L63/Ile mutant has a torsional energy of 782.5 Kcal/mol, the steric effect induced by presence of the two Ile residues decreasing probably, the enzyme flexibility. We suggest that for the Val82/Asp mutant (882.3 Kcal/mol) the increase of enzyme flexibility is determined by the presence of Asp residue (Fig. 2c).

An unexpected flexibility was recorded for the Ala71/Thr, Val82/Ala mutant (847.4 Kcal/mol) which gains a heavy group from the Thr residue and loses the methyl group from the Val residue. We suppose that the flexibility could be induced by the substitution of Ala residue in the position 82.

Solvent accessible surface for mutants and wild type viral protease cavity

After computing the solvent accessible surfaces of the HIV-1 PR wild type and mutants we noticed that the HIV-1 PR mutants have a solvent accessible protease active site cavity surface either higher (Cys95/Ala; Val82/Phe; Val82/Phe, Ile84/Val; Val82/Phe, Cys95/Ala) or significantly lower (Val82/Asn; Leu33/Ile, Cys67/ABA, Cys95/ABA; Ile84/Val; Val82/Ala; Gln7/Leu, Leu33/Ile, Leu63/Ile; Val82/Asp; Gly48/His) than the wild type (Table 2).

The localization of the substituted aminoacids is shown in Fig. 3 (using HyperChem5 software, http://www.hyper.com/products/, Hypercube, Inc., Gainesville, Fl.32601, USA) [34]. By SAS calculation we noticed that the Val82/Phe mutant (the 82 residue is localized more closely to the active site) had the highest SAS value (2477.0 Å^2), while the HIV-1 PR wild type has a SAS of 2380.2 Å^2, only. We can explain the solvent accessible surface difference between the Val82/Phe mutant and the wild type, by the steric repulsive interaction between the benzyl group from Phe residue and the Ile84 and Pro81 residues situated very closely to the 82 residue. We suppose that the repulsive steric interaction can changes the spatial arrangement of the benzyl group, which will be pushed outside the protease active site cavity.

Even more, if the SAS for Val82/Asp; Val82/Ala and Val82/Asn mutants are analyzed, we noticed a decrease of the SAS (Val82/Asp = 2127.5 Å^2; Val82/Ala = 2288.6 Å^2; Val82/Asn = 2302.8 Å^2). We explained the SAS decrease for the Val82/Asp and Val82/Asn by the presence in the neighborhood of the protease active site cavity of heavy groups (carboxy and amino) belonging to Asp and Asn residues.

Other important structure, which has the changed position close to the active site, is the Ile84/Val mutant. The decrease of the mutant SAS (Ile84/Val = 2292.3 Å^2) can be explained by the presence of two very close Val residues (84 and 82) which have the metylen groups orientated inside the cavity. For the Gly48/His mutant, the imidazolyl group is capable to induce a steric repulsion with the two Ile residues (47,50) situated very close to the His48 and the active site. The Cys95/Ala mutant (SAS: Cys95/Ala = 2463.6 Å^2) is also interesting. We suppose that the molecular modifications of the spatial configuration induced by the Ala residue can produce the opening of the protease active site cavity. An important step in our study was the SAS analysis for the double mutants. We calculated the SAS for four HIV-1 PR double mutants (Val82/Phe, Cys95/Ala = 2443.5 Å^2; Ile84/Val, Cys95/Ala = 2391.9 Å^2, Val 82/Phe, Ile 84/Val = 2466.0 Å^2 and Ala71/Thr,Val 82/Ala = 2301.1 Å^2). We have choose these mutants because they contain one mutation close to the active site (e.g.82 and 84 residue positions) and one away from the active site (*e.g.* 95 or 71 residue positions) except the Val82/Phe, Ile84/Val mutant. We noticed that all mutants with the Val82/Phe mutation have a high solvent accessible surface when compared to the wild type, but a lower solvent accessible surface when compared to HIV-1 PR Val82/Phe mutant (see Table 2). When the HIV-1 PR Val82/Phe, Ile84/Val mutant is considered, we can suggest that the high solvent accessible surface (2466.0 Å^2) is induced by the presence of steric repulsive interactions between the Val84 and Phe82 residues.

The same explanation is given for the Ile84/Val, Cys95/Ala mutant, which has a low SAS compared to the Cys95/Ala mutant. It is difficult to explain the solvent accessible surface decrease for the Val 82/Phe, Cys95/Ala because both single mutations have a higher SAS than Val82/Phe, Cys 95/Ala. In

these conditions we can consider that the presence of mutation do not affect in the same manner the protease active site cavity opening. For the triple and multiple mutations we have found a decrease of the solvent accessible viral protease cavity surface. We suggest that the solvent accessible surface represented a useful parameter when the HIV-1 PR mutants resistance mechanism develops for various pharmacological agents is analyzed.

Discussions

Even if, the efforts to design drugs for the inhibition of HIV-1 PR are important, the capability of the protease to produce the mutant structures represent a serious problem for the anti-AIDS therapy [6]. The high mutation rate of HIV-1 protease imposes the testing of a lot of inhibitors, which belong to very different chemical classes. In the last years, a special attention was given to the cyclic urea derivatives [2, 6, 23, 35] and the ritonavir derivatives [2, 6, 36-38]. Analysis of the HIV-1 PR gene from the plasma of HIV-1 infected patients revealed a large number of mutations (nine) during the monotherapy with the protease inhibitors, especially, the cyclic urea derivatives [2] (DMP323, DMP450, SD146), and ritonavir [36-38]. The Val82/Phe, Val82/Ala, Ile84/Val and Val82/Phe, Ile84/Val mutations often appeared in most patients [2]. The single mutations Val82/Phe and Ile84/Val caused changes with different inhibitors included saquinavir, indinavir, nelfinavir, ritonvir and 14 cyclic ureas, ranging from 0.3- to 86- fold in K_i and from 0.1- to 11-fold in IC_{90}.

The critical viral resistant to saquinavir, indinavir, nelfinavir, ritonvir and 14 cyclic ureas inhibitors develops the double mutation Val82/Phe,Ile84/Val (Ki from 10-2000-fold) [2, 36-38].
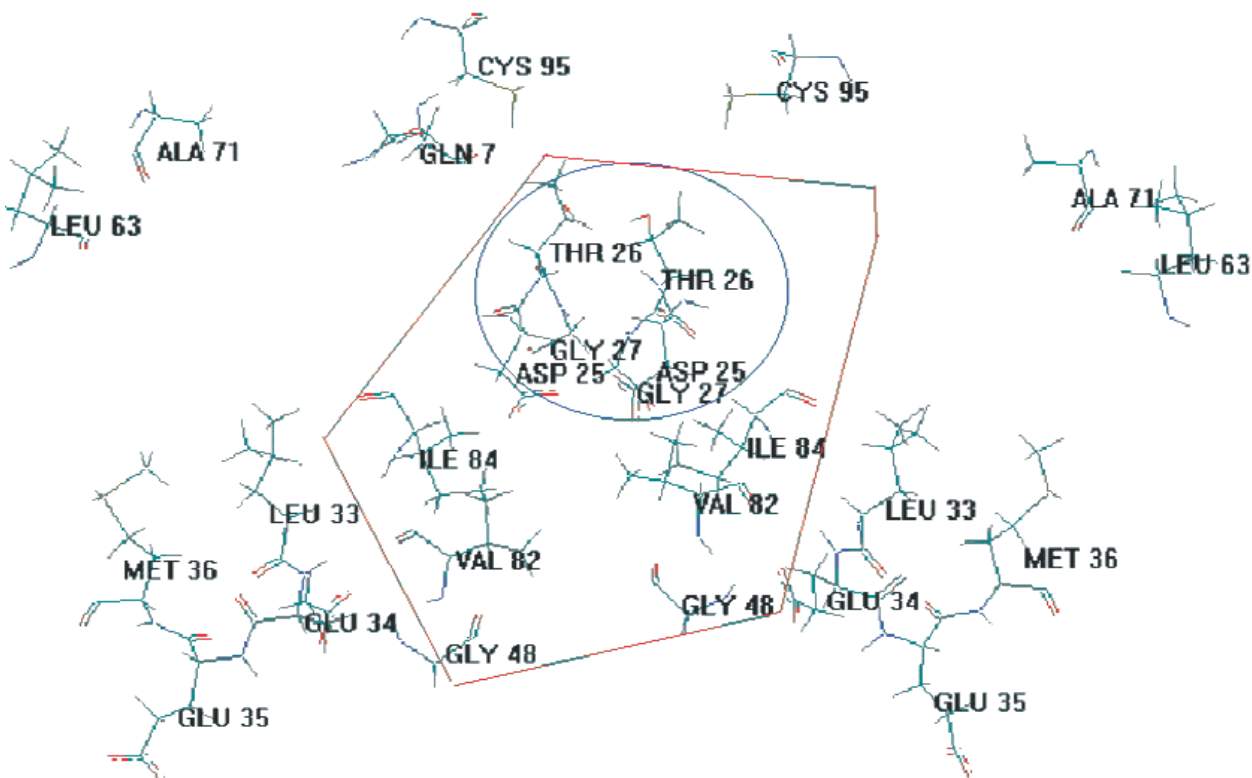

Fig. 3 The spatial localization of the substituted aminoacids compared to the HIV-1 PR active site. The residues number 31, 33, 36, 35, 63, 71, 95 are localized away from the active site while the residues number 48, 82, 84 are situated very closely to the HIV-1 PR active site. blue – isolated aria of the active site(residues number 25,26,27), red- the isolated aria contains a number of three residues, 48, 82,84, belonging to viral protease cavity (e.g. residues number 48, 82,84 are situated very closely to the HIV-1 PR active site). - worked in HyperChem5 software, Hypercube, Inc. 1115 NW 4th Street, Gainesville, FL 32601 USA, 2001, http://www.hyper.com.

In our study we suggest that the HIV-1 protease mutations are capable to induce (i) important changes of the torsional energy of the HIV-1 PR and (ii) changes of the solvent accessible cavity surface of the virus. We noticed a different energetic (torsional and van der Waals) effect induced by the Ile and Lys residues) in the Gln7/Leu, Leu33/Ile, Leu63/Ile mutant compared to the wild type HIV-1 protease (see Fig. 2). While the Ile and Lys residues do not produce a significant difference between the electrostatic force fields of the wild type and the triple mutant, the van der Waals plot present a large difference (Fig. 2). We concluded that the presence of hydrophobic groups (e.g. methylen groups) could be responsible for the increase of the mutant van der Wals energy. On the other hand, if the triple mutant flexibility is analyzed, one can notice that the presence of the Ile and Lys residues are capable to induce a low flexibility on the whole enzyme structure, even if the solvent accessible surface of the protease active site cavity is higher. This observation give us the possibility to conclude that a study of the viral mutant resistance impose a complete analysis of the torsional energy of the whole mutant enzyme and, in the same time, the torsional relaxation analysis of the active site belonging to the mutant.

Analyzing the electrostatic energy for the mutant Ala71/Thr, Val82/Ala we can conclude that the presence of the hydroxyl group from the Thr residue is not capable to induce an important modification of the mutant electrostatic force field. This conclusion can be uphold by the spatial distribution of the HIV-1 protease peptide chains, which imposes a large distance between the protease active site and the position 71 where the Thr residue is present. The unfavorable steric effect (induced by losing of the methyl group) in the 82 protease chain position, which is situated very closely to the active site, is reduced by the elimination of the methyl group. Loosing the methyl group from the 82 position gives an increased flexibility to the mutant protease compared to the wild type protease.

For the energetic study of the Val82/Asp and Val82/Asn mutants we expected to obtain a small energetic difference between mutants. By displaying the energetic plots we observed that, the mutations having the same position do not induce a change of the electrostatic interactions or the torsion energy, while the van der Waals intramolecular contacts were disturbed. Of course, the multiple mutants for which the substituted positions are localized far away or closely to the active site (see torsion energy of the Gln7/Lys, Leu33/Ile, Leu63/Ile mutant, solvent accessible surface of Val82/Phe, Cys95/Ala mutant (2413.5Å^2), Val 82/Phe, Ile84/Val mutant (2466 Å^2)) have a great importance for our study. The spatial localization of the mutations is important, especially, when a mutant aminoacid affects the interactions between the HIV-1 protease active site and the substrate or the inhibitors, directly through steric, electrostatic or hydrophobic interactions or indirectly by conformational modifications. It is possible to consider that the presence of heavy atoms or of a larger number of aliphatic groups can induce important steric effects that can induce a decrease or increase of the binding capacity of inhibitors. Of course, in this condition we must consider the chemical nature of the inhibitor, more exactly, the solvent accessible surface and molecular volume of the inhibitors. It is very advantageous to obtain solvent accessible surfaces for all mutants and inhibitor structures.

That gives the possibility to display the electrostatic potential and lipophilic potential on the surface of the HIV-1 PR mutants and to try a comparative study of the geometric conformation of the chemical structures. These are important in drug design. Ideally, inhibitors should be designed to interact with residues that are essential for protease activity, because the resistant variants would produce an inactive enzyme. In our study we show that the simple (Val82/Phe = 2477 Å^2) and double (Val82/Phe, Ile84/Val = 2466 Å^2) mutations are capable to induce a large HIV-1 viral protease cavity which can produce a significant modification of the active site interaction with the viral inhibitors. We conclude that all parameters (i) the number of mutations; (ii) the place of mutation and (iii) the steric interactions, are very important for the analysis of the interaction among HIV-1 PR mutants and their inhibitors. With our results we show that, mainly, the mutations situated close to the protease active site cavity, are capable to change the solvent accessible cavity surface. The essential effect involved in this modification is the steric repulsive effect induced by the heavy atoms (metylen or imidazolyl groups).

When the double mutations were analyzed, we found that the solvent accessible surface decreased

even if, the mutant position are close to the active site (Val82/Phe, Ile84/Val = 2466 $Å^2$ versus Val82/Phe = 2477 $Å^2$ or Ile 84/Val, Cys95/Ala versus Cys95/Ala). The multiple mutations produce a decrease of the SAS, probably induced, by multiple spatial changes in the HIV-1 PR configuration chains.

The results show that the mutations presence, even if, the amino acids substitution are away from the active site, can induces a modification of the energetic parameters (van der Waals energy, electrostatic energy and torsional energy) and of the solvent accessible surface of the protease active site cavity. The amino acids substitution can induce a different enzymatic inhibition as in the HIV-1 protease wild type. All these changes are possible explanations for the generation of the mutant HIV-1 PR drug resistance.

Acknowledgements

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