Biochemical and Biophysical Research Communications 430 (2013) 1022–1027

Contents lists available at SciVerse ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Conserved hydrogen bonds and water molecules in MDR HIV-1 protease substrate complexes

Zhigang Liu [a,b,c], Yong Wang [a], Ravikiran S. Yedidi [a,d], Tamaria G. Dewdney [a], Samuel J. Reiter [a], Joseph S. Brunzelle [e], Iulia A. Kovari [a], Ladislau C. Kovari [a,*]

[a] Department of Biochemistry and Molecular Biology, Wayne State University School of Medicine Detroit, MI 48201, United States
[b] Department of Pathology, Case Western Reserve University Cleveland, OH 44106, United States
[c] Division of Internal Medicine, Harbor Hospital Baltimore, MD 21225, United States
[d] Experimental Retrovirology Section, HIV and AIDS Malignancy Branch, National Cancer Institute, National Institutes of Health, Bethesda, MD 20892, United States
[e] Life Sciences Collaborative Access Team and Department of Molecular Pharmacology and Biological Chemistry Northwestern University Feinberg School of Medicine Chicago, IL 60611, United States

ARTICLE INFO

Article history:
Received 28 November 2012
Available online 19 December 2012

Keywords:
HIV-1 protease
Multi-drug resistance
Crystallographic structure
Protease-substrate complex
Conserve hydrogen bonds and waters

ABSTRACT

The success of highly active antiretroviral therapy (HAART) in anti-HIV therapy is severely compromised by the rapidly developing drug resistance. HIV-1 protease inhibitors, part of HAART, are losing their potency and efficacy in inhibiting the target. Multi-drug resistant (MDR) 769 HIV-1 protease (resistant mutations at residues 10, 36, 46, 54, 62, 63, 71, 82, 84, 90) was selected for the present study to understand the binding to its natural substrates. The nine crystal structures of MDR769 HIV-1 protease substrate hepta-peptide complexes were analyzed in order to reveal the conserved structural elements for the purpose of drug design against MDR HIV-1 protease. Our structural studies demonstrated that highly conserved hydrogen bonds between the protease and substrate peptides, together with the conserved crystallographic water molecules, played a crucial role in the substrate recognition, substrate stabilization and protease stabilization. In addition, the absence of the key flap-ligand bridging water molecule might imply a different catalytic mechanism of MDR769 HIV-1 protease compared to that of wild type (WT) HIV-1 protease.

1. Introduction

The current treatment of choice to suppress *in vivo* HIV replication is highly active antiretroviral therapy (HAART) since the mid 199 [1–3]. However, neither a cure nor vaccine is available for HIV infection [4,5]. Three or more drugs are combined in HAART regimen, for example two nucleoside reverse transcriptase inhibitors (NRTIs) and one non-nucleoside reverse transcriptase inhibitor (NNRTIs) or one protease inhibitor (PI). Despite the success of HAART, the drug resistance is emerging rapidly as a result of the absence of proofreading function of reverse transcriptase [6–8], which makes it urgent to develop novel drugs to combat against the drug resistance [9–13].

HIV-1 protease (HIV PR) is an aspartic protease, a symmetric homodimer with 99 amino acids in each monomer [14], that cleaves newly synthesized HIV-1 polyproteins (nine major cleavage sites) to generate the mature protein components of an infectious HIV-1 virion. The HIV-1 *gag* gene codes for structural proteins: matrix protein (MA), capsid protein (CA), and nucleocapsid protein (NC) while the *gag-pol* gene encodes both structural proteins (MA, CA and NC) and enzymes such as: protease (PR), reverse transcriptase (RT), RNAse H (RH), and integrase (IN). Without effective HIV-1 PR, HIV-1 virions remain uninfectious, hence making HIV-1 protease inhibitors the most potent anti-HIV drugs and essential therapeutic components of HAART [1,11].

Many crystal structures are available in literature with WT HIV-1 protease or limited drug resistant HIV-1 mutants complexed with various substrate peptides [15–20]. The analysis of these structures demonstrated a conserved asymmetric binding pattern of different substrate peptides with various sequences. Nonetheless, the crystallographic information is limited analyzing the binding pattern of the nine substrate peptides to MDR HIV-1 protease.

To understand the structural characteristics of MDR HIV-1 protease substrate peptide complexes, we chose a clinical isolate,

Abbreviations: MDR, multi-drug resistant; HIV, human immunodeficiency virus; WT, wild type; HAART, highly active antiretroviral therapy; PR, protease; MA, matrix; CA, capsid; NC, nucleocapsid; RT, reverse transcriptase; IN, integrase; RH, rNase H; RMSD, root mean square deviation.

* Corresponding author. Address: 540 E. Canfield Avenue, 4263 Scott Hall, Detroit, MI 48201, United States.
E-mail address: kovari@med.wayne.edu (L.C. Kovari).

0006-291X/$ - see front matter © 2012 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.bbrc.2012.12.045

MDR769 HIV-1 protease, as our study model. The high resolution crystal structure of MDR769 HIV-1 protease was solved by our group and it showed an expanded active site cavity with mutations at positions 10, 36, 46, 54, 62, 63, 71, 82, 84, 90 [21–23]. The nine hepta-peptides corresponding to the natural cleavage sites P3 to P4′ were co-crystallized with inactive MDR769 HIV-1 protease and the structures were reported recently [24].

Further detailed structural analysis revealed that the highly conserved hydrogen bonds between the protease and substrate hepta-peptides, together with the conserved crystallographic water molecules, played a crucial role in the substrate recognition, substrate stabilization and the protease stabilization. In addition, the absence of the key flap-ligand bridging water molecule might imply a different catalytic mechanism of MDR769 HIV-1 protease compared to that of wild type (WT) HIV-1 protease. All these findings may provide a novel strategy to design HIV-1 protease inhibitors with excellent resistance profiles.

2. Materials and methods

2.1. Substrate peptides preparation

The nine substrate hepta-peptides were purchased from SynBioSci Corporation, Livermore, CA (Table 1). All the hepta-peptides were purified by HPLC to purity higher than 98%. Peptide powder was dissolved in DMSO to prepare stock solution of 20 mM concentration and the samples were stored at −20 °C.

2.2. Protein purification and co-crystallization

The MDR769 HIV-1 A82T protease was over expressed by using a T7 promoter expression vector in conjunction with the *Escherichia coli* host, BL21 (DE3). Details of protein expression and purification were discussed in our previous research [22,24].

The substrate hepta-peptides were mixed with 2.5 mg/ml MDR769 HIV-1 A82T and diluted to 0.4 mM final concentration. The hanging drop vapor diffusion method was used to form the bi-pyramidal crystals of the MDR769 protease, with crystallization conditions published before [24].

2.3. Data collection and crystallographic refinement

Protease crystals were dipped in 30% glucose for cryoprotection and flash frozen in liquid nitrogen. The diffraction data were collected at 1.00 Å wavelength at the Advanced Photon Source (APS) (LS-CAT 21), Argonne National Laboratory (Argonne, IL). Data were reduced to structure amplitudes with CrystalClear (CrystalClear: An Integrated Program for the Collection and Processing of Area Detector Data, Rigaku Corporation, 1997–2002). In all cases the crystals belonged to the same space group *P41*. Molecular replacement was performed with Molrep-autoMR in CCP4 [25] with model previously solved in our lab [26]. Initial refinements were performed without substrate hepta-peptides using Refmac5 [27,28]. The nine hepta-peptides were built into the difference electron density maps as the refinement processed in program COOT [29]. Crystallographic waters were added with program ARP/wARP [30]. The structures were refined to resolution 1.6–2.0 Å in Refmac5. The final stereochemical parameters were checked using PROCHECK [31]. Images were generated in PyMol (DeLano, W.L. The PyMOL Molecular Graphics System (2002) DeLano Scientific, Palo Alto, CA, USA.)

Table 1
Sequences of the nine sites within the HIV-1 Gag and Pol polyproteins that are cleaved by HIV-1 protease.

Cleavage site	P3	P2	P1	P1′	P2′	P3′	P4′	PDB code
MA/CA	Gln	Asn	Thr	Pro	Ile	Val	Gln	3OTS
CA/p2	Arg	Val	Leu	Phe	Glu	Ala	Met	3OUD
p2/NC	Thr	Ile	Met	Met	Gln	Arg	Gly	3OUC
NC/p1	Gln	Ala	Asn	Phe	Leu	Gly	Lys	3OUB
p1/p6	Gly	Asn	Phe	Leu	Gln	Ser	Arg	3OUA
TF/PR	Phe	Asn	Phe	Pro	Gln	Ile	Thr	3OU4
PR/RT	Leu	Asn	Phe	Pro	Ile	Ser	Pro	3OU3
RT/RH	Glu	Thr	Phe	Tyr	Val	Asp	Gly	3OTY
RH/IN	Lys	Val	Leu	Phe	Leu	Asp	Gly	3OU1

The cleavage sites are named by the proteins released after the sites are cleaved: matrix (MA), capsid (CA), nucleocapsid (NC), trans frame peptide (TF), protease (PR), reverse transcriptase (RT), RNAse H (RH), and integrase (IN).

2.4. Analysis

Hydrogen bonds docking hepta-peptides were analyzed by Pisa Server [32]. Conserved hydrogen bonds within protease were identified with program Ligplot [33]. Temperature factor analysis was done with the CCP4 program Temperature Factor Analysis. Protease substrate complexes were superimposed based on protease residues 1 to 99 Cα and RMSD of Cα was analyzed with the CCP4 program Superpose Molecules [34]. Solvent accessible area was calculated with the CCP4 program Accessible Surface Areas. Water molecules located within 2.0 Å among nine complex structures were considered conserved. The criterion was extended to 3 Å if the water molecules formed similar interaction pattern among nine complexes. All analysis was visualized using PyMol.

3. Results

3.1. Determination of crystal structures of MDR769 HIV-1 protease-substrate complexes

The inactive MDR769 HIV-1 protease was co-crystallized with nine hepta-peptides representing the nine natural substrate cleavage site sequences in Gag and Gag-Pol polyproteins. These complexes crystallized into space group *P41*, in which each asymmetric unit contained one biologically relevant HIV-1 protease dimer complexed with one ligand. The structures were refined to 1.6–2.0 Å resolution. The hepta-peptides were named after the proteins released upon cleavage: matrix (MA), capsid (CA), nucleocapsid (NC), trans frame peptide (TF), protease (PR), reverse transcriptase (RT), RNAse H (RH), and integrase (IN).IHIV The sequence of these peptides and the PDB accession codes are shown in Table 1.

3.2. Ligand backbone specific hydrogen bonds

The ligand backbone specific hydrogen bonds refer to the hydrogen bonds connecting the substrate backbone atoms and the protease atoms, which mimic the conformation of β strands. They are either conserved (present in at least six complexes out of nine) or non-conserved (present in less than six complexes out of nine) hydrogen bonds among the nine protease substrate complexes as demonstrated in Table 2 and Fig. 1.

Compared to the WT HIV-1 protease substrate complexes, the amount of both conserved and non-conserved ligand backbone specific hydrogen bonds are reduced, which may be one of the major reasons of weaker substrate binding to the MDR HIV-1 protease.

Six conserved ligand backbone specific hydrogen bonds are observed; Table 2A shows the residues involved in these hydrogen bonds, which bridge the backbone atoms of hepta-peptides at sites P3, P1, P2′ and the atoms from protease residues Asn 25, Gly 27 and Asp 29.

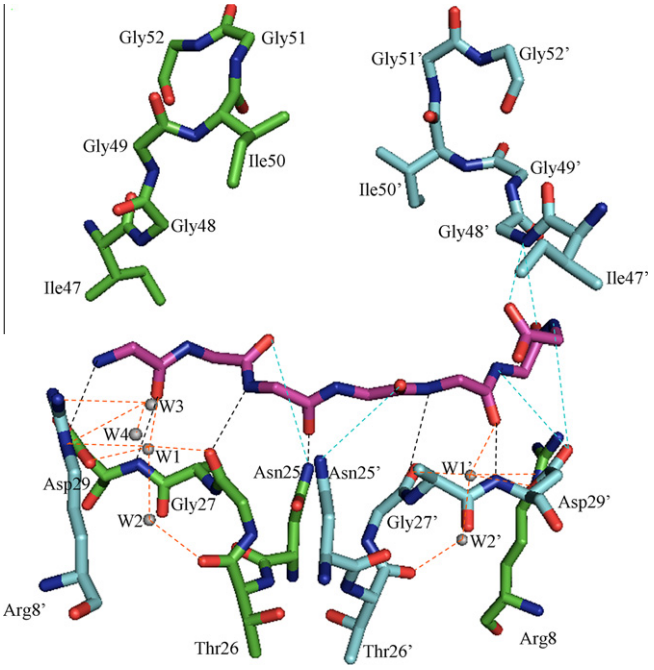

Fig. 1. Ligand backbone specific hydrogen bonds and crystallographic waters involved in substrate peptide recognition. The substrate backbone was shown in purple with stick model, and the MDR HIV-1 protease monomers are shown in green and cyanosis with stick model. The crystallographic water molecules are shown with grey sphere model. The conserved ligand backbone specific hydrogen bond and non-conserved ligand backbone specific hydrogen bonds between the substrate peptide and MDR HIV-1 protease are presented with black and cyanotic dash line respectively. The hydrogen bonds mediating substrate recognition via crystallographic water molecules are shown with orange dash line.

Table 2a
Conserved ligand backbone specific (substrate peptide) hydrogen bonds and length (Å).

Substrate atom	Protein atom	MA/CA	CA/p2	p2/NC	NC/p1	p1/p6	TF/PR	PR/RT	RT/RH	RH/IN
P3 O	Asp 29 N	2.8	2.87	2.94	3.13	2.73	2.81	2.75		
P3 N	Asp 29 OD1	2.57	2.73		2.81			2.49	2.58	3.2
P1 O	Asn 25 ND2	3.02	A 3.42B 3.13		3.11	2.49		3.00		A 2.89B 3.18
P1 N	Gly 27 O	3.51	3.42	3.2	3.26	3.1	3.71	3.49	2.90	3.01
P2′ O	Asp 29 N	3.16		3.3	2.96	2.73	3.15		3.13	2.90
P2′ N	Gly 27 O	2.93	3.14	3.13	3.55	2.96	2.83	2.85	2.83	3.04

The non-conserved ligand backbone specific hydrogen bonds are present between the substrate backbone and the protease residues Asn 25, Asp 29', Gly 48'. The oxygen atom of p1/p6 C-terminal (OXT) forms a hydrogen bond with Gly 48'. Notably, in NC/p1, the nitrogen atom at P4′ forms a hydrogen bond with carbonyl oxygen of Gly 48 instead of residue Asp 29' (Table 2B). P2 and P1' sites lack both conserved and non-conserved ligand backbone specific hydrogen bonds. P1′ backbone atoms form hydrogen bonds in RT/RH and RH/IN complexes, but the length of these hydrogen bonds are too long to contribute significantly to the interaction. Only CA/p2 forms a hydrogen bond between P2 carbonyl oxygen atom and the protease atom Asn 25 ND2 (Table 2B).

3.3. Ligand side chain specific hydrogen bonds

Besides fewer hydrogen bonds involving substrate peptide backbone atoms, fewer hydrogen bonds arising from side chains of the substrate peptides may also weaken the binding for ligands to MDR HIV-1 protease (Table 3). With the exception of NC/p1, all the hepta-peptides are docked to the protease by two to six ligand side chain specific hydrogen bonds. Most of the side chain specific hydrogen bonds (24 out of 29 hydrogen bonds in all nine complexes) involve side chains of P3, P2, and P2′, whereas P1, P1′, P3′ and P4′ form fewer hydrogen bonds (only 5 hydrogen bonds). On the protease, both the side chains of Arg 8, Asp 30, Thr 82, Asp 25 and the backbones of Asp 29, Asp 30, Pro 81, Leu 46, Gly 48 are involved in hydrogen bonds with hepta-peptide side chain atoms. In addition, in CA/p2 complex, Arg 1 NH1 (P3) forms hydrogen bond with Thr 82 OG1, due to the A82T mutation.

3.4. The conserved flap-ligand bridging water reported for the wild type protease is missing in the MDR-ligand complex

The flap-ligand bridging water molecule is missing, while it is conserved in WT HIV-1 protease substrate peptide complexes tethering the protease flap (Ile 50 N and Ile 50' N) with ligands (P2 O and P1′O). This missing water molecule plays a crucial role in substrate cleavage by forming a transitional molecule in the WT HIV-1 protease substrate complexes; in addition, it contributes to the substrate recognition and stabilization in the WT HIV-1 protease substrate complexes. Without this key water molecule, the binding of substrate peptide to MDR769 HIV-1 protease is weakened and cleavage mechanism of the substrates may be different. As a result, HIV-1 protease inhibitors based on different mechanism may be required to overcome the drug resistance imposed by MDR769 HIV-1 protease.

3.5. The conserved water network contributes to the recognition and stability of the ligands in MDR protease substrate complexes

Eleven highly conserved water molecules are found in the MDR HIV-1 protease active site cavity with different functions in substrate recognition, five of which are also observed in WT HIV-1 protease substrate complexes. It is not surprising to have more conserved water molecules in the active site cavity of MDR HIV-1 protease substrate complexes, considering its expanded active site cavity and substrate envelope. Based on their functions, the water molecules fall into five categories: I. Directly bridge substrate and

Table 2b
Non-conserved ligand backbone specific (substrate peptide) hydrogen bonds and length (Å).

P2 O	Asn 25 ND2	2.64								
P2 N										
P1′ O	Asn 25 ND2								3.68	3.79
P1′ N										
P3′ O	Gly 48′ N	2.96					3.49		3.13	
P3′ N	Asp 29′ OD1	3.86								
P4′ O	Gly 48′ N	3.66		2.83	2.97, OXT 2.89[b]					
P4′ N	Asp 29′ OD1		2.66	3.76 (Gly 48 O)[a]	3.23					2.41

[a] P4′ N of NC/p1 forms hydrogen bond with Gly 48 O instead of Asp 29 OD1.
[b] P4′O of p1/p6 forms hydrogen bond with both Gly 48 N and OXT.

Table 3
Observed side chain (substrate peptide) to side chain (protease) and side chain (substrate peptide) to backbone (protease) hydrogen bonds.

Cleavage site	Side chain to side chain interaction			Side chain to backbone interaction		
	Substrate atom	Protein atom	Length (Å)	Substrate atom	Protein atom	Length (Å)
MA/CA	Gln 1 OE1	Arg 8 NH2	3.07	Asn 2 OD1	Asp 29 N	3.12
	Asn 2 ND2	Asp 30 OD1	3.79	Asn 2 OD1	Asp 30 N	2.94
CA/p2	Arg 1 NH1	Thr 82 OG1	3.35	Arg 1 NH1	Pro 81 O	3.26
				Glu 5 OE1	Asp 29 N	3.29
				Glu 5 OE1	Asp 30 N	2.96
p2/NC	Met 3 SD	Asn 25 ND2	3.5	Gln 5 NE2	Asp 30 O	3.80
NC/p1						
p1/p6	Gln 5 NE2	Asp 30 OD1	3.62	Arg 7 NE	Leu 46 O	2.68
	Asn 2 OD1	Asn 25 ND2	3.5			
TF/PR	Gln 5 NE2	Asp 30 OD1	3.0	Gln 5 OE1	Asp 29 N	2.93
	Thr 7 OG1	Asp 30 OD1	2.92	Gln 5 OE1	Asp 30 N	2.90
				Asn 2 OD1	Asp 29 N	3.14
				Asn 2 OD1	Asp 30 N	3.08
PR/RT	Asn 2 ND2	Asp 30 OD1	3.85	Asn 2 OD1	Asp 29 N	2.95
				Asn 2 OD1	Asp 30 N	2.92
RT/RH	Glu 1 OE2	Arg 8 NH1	3.17	Thr 2 OG1	Asp 30 O	3.87
	Asp 6 OD2	Arg 8 NH2	2.54	Thr 2 OG1	Asp 29 N	3.20
				Thr 2 OG1	Asp 30 N	3.22
RH/IN	Asp 6 OD1	Arg 8 NH2	2.76	Lys 1 NZ	Gly 48 O	3.00

protease; II. Directly and indirectly bridge substrate and protease; III. Indirectly bridge substrate and protease; IV. Stabilize the water network in protease active site cavity; V. Fill the space without obvious functional indication. First, W1 /W1′ (symmetric) connect P3O/P2′O with Gly 27 O, Asp 29 OD2, Arg 8' NE/Gly 27' O, Asp 29' OD2, and Arg 8 NE respectively. Second, W3 (asymmetric) connects W1 directly to protease at residue Arg 8' NE and Arg 8' NH1. But the finding that W3 also connects directly to hepta-peptides in MA/CA, CA/p2, NC/p1, TF/PR, and RH/IN complexes renders W3 the capacity to stabilize directly the hepta-peptides conformation. Third, W2/W2′ (symmetric) form hydrogen bonds with W1/W1′ and Thr 26 O, Arg 87 NE/Thr 26' O, Arg 87' NE respectively. Fourth, W4 (symmetric to W5) connects to W3, stabilizing W3. In contrast, in MA/CA, NC/p1, p1/p6, TF/PR, and RT/RH complexes, W4 connects to protease at Thr 82' OG1, showing the significance of the mutation A82T in substrate recognition. Fifth, W5, W6, W7, W8, W9

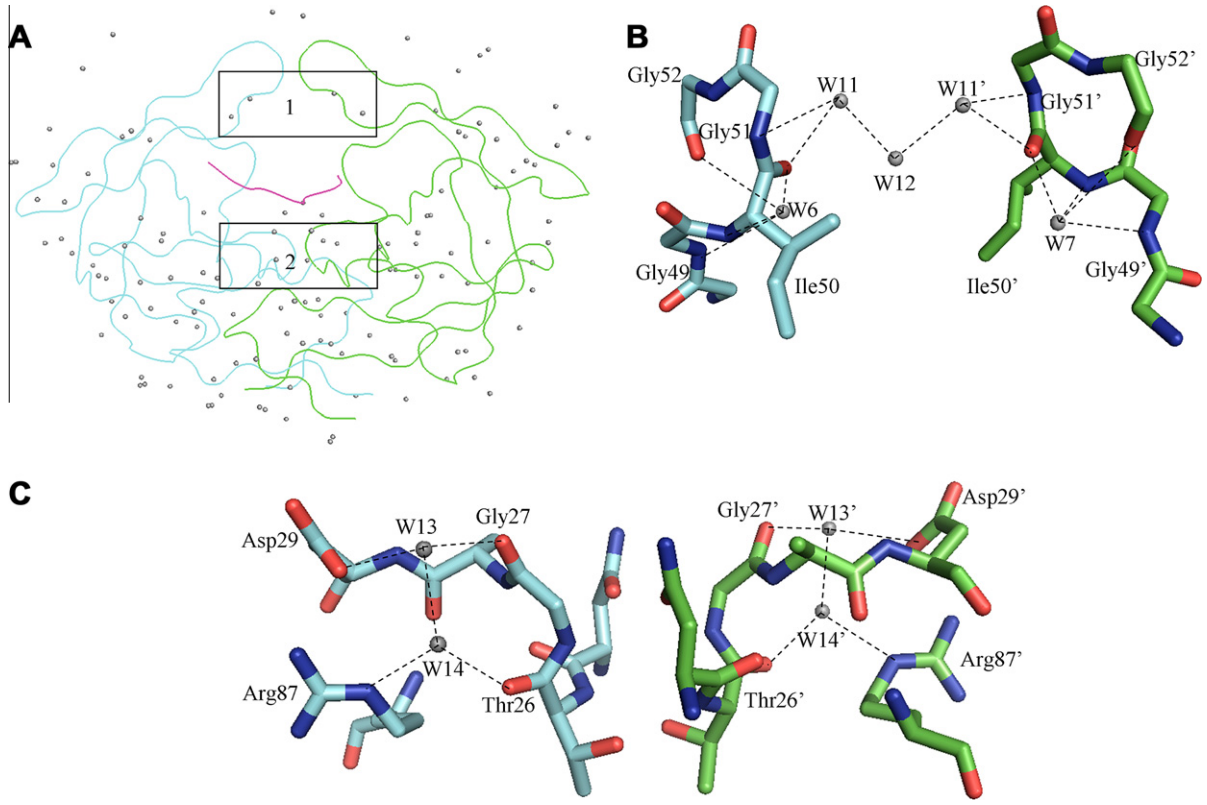

Fig. 2. Conserved crystallographic water molecules among the nine MDR HIV-1 protease substrate complexes. Panel A. Overview of the highly conserved crystallographic water molecules. Grey spheres represent the crystallographic water molecules observed in all the nine complexes. The MDR HIV-1 protease monomers and substrate peptide are shown with cyanotic, green and purple ribbons respectively. The box 1 and box 2 are in the flap region and active site cavity which is investigated in more details in panel B and C. Panel B. Detailed analysis of the crystallographic water molecules in the flap region. Panel C. Detailed analysis of the crystallographic water molecules in the active site cavity.

connect only to protease and seem to fill the space only without significant functional role in substrate recognition. W5, W6, W7, W8, and W9 are far away from hepta-peptides, but reside within the active site cavity. Fig. 1 presents the conserved water molecules from the first four categories based on their function.

3.6. The conserved water network contributes to the stability of the MDR HIV-1 proteases

Along with the highly conserved hydrogen bonds are the highly conserved crystallographic water molecules among the nine protease-substrate complexes. 193–299 crystallographic water molecules are identified in each of the nine complexes, among which 125 are completely conserved (Fig. 2A).

Among the 125 conserved water molecules, 52 form at least two hydrogen bonds with protease residues, 43 are on the surface of the protease forming only one hydrogen bond with protease residues, 12 are on the surface of the flaps, 6 are involved in substrate recognition. In addition, 12 water molecules are on the surface of the protease without direct interaction with protease, instead they interact with other fixed water molecules to form a water network.

Among the 125 completely conserved water molecules, 114 are twofold symmetrically distributed in the dimer. The distribution of these asymmetric water molecules are described as the following. Two are asymmetrically distributed at the dimerization interface, one is involved in the substrate binding, three are on the flap area, three are around residues 60–67, one is between residues 61 and 40, and one is around the α helix. Of these 11 asymmetric water molecules, six are on one monomer, four are on the other, and one is between two monomers.

Conserved water molecules are crucial in the tertiary structure stabilization. First, in both monomers, W10/W10′ bridge the carbonyl oxygen of Val 78/Val 78' (preceding 80s loop) with NH1 and NE of Arg 57/Arg 57' (downstream the flap area). These water molecules fix the N terminus of 80 s loop and the C terminus of the flap, which are crucial in the substrate recognition and binding. Second, Ile 50 O, Ile 50 N, Gly 49 N and Gly 52 O in each monomer are connected through a hydrogen bond network contributed by the conserved water molecules W6/W7. The internal hydrogen bonds by W6/W7 stabilize the conformation of the flap tips (Fig. 2B). Third, water network W11, W11′ and W12 is important in the interaction between the flap tips from different monomers, although the flap tips do not interact directly. W12 is conserved in eight complexes with one exception RH/IN; W11 is conserved in seven complexes, while W11′ is present in six complexes. The three water molecules form an isosceles triangle with W12 in the middle pointing down to the active site cavity. W12 forms indirect hydrogen bonds with the protease through W11/ W11′ water molecules, which connect directly to Ile 50/50' O and Gly 51/51' N respectively. Thus the water network actually links the tips of the flaps together (Fig. 2B). Fourth, the active site loop is also stabilized by water molecules. W13 /W13′ bridge Gly 27 O/ Gly 27' O with Asp 29 OD2/ Asp 29' OD2 and W14/W14′ bridge Thr 26 O/Thr 26' O with Arg 87 NE/Arg 87' NE. Fifth, on the dimerization interface, six conserved water molecules connect the two monomers together. W15/W15′ connect together Asn 98' O, Gly 94 O, Thr 96 OG1/Asn 98 O, Gly 94' O, Thr 96' OG1 respectively. W16/W16′ connect together Cys 95 O, Leu 5' N/Cys 95' O, Leu 5 N respectively. W17/W17′ connect together Ile 93 O, Phe 99'OXT/Ile 93' O, Phe 99 OXT respectively (Fig. 3). Sixth, there are four conserved water molecules that stabilize the N terminal loop around the dimerization area: W18/W18′ connect together Pro 9 N, Gln 7 O, Thr 4 O/Pro 9' N, Gln 7' O, Thr 4' O respectively, and W19/W19′ connect together Gln 7 N, Trp 6 N, Thr 4 O/Gln 7' N, Trp 6' N, Thr 4' O respectively (Fig. 3).

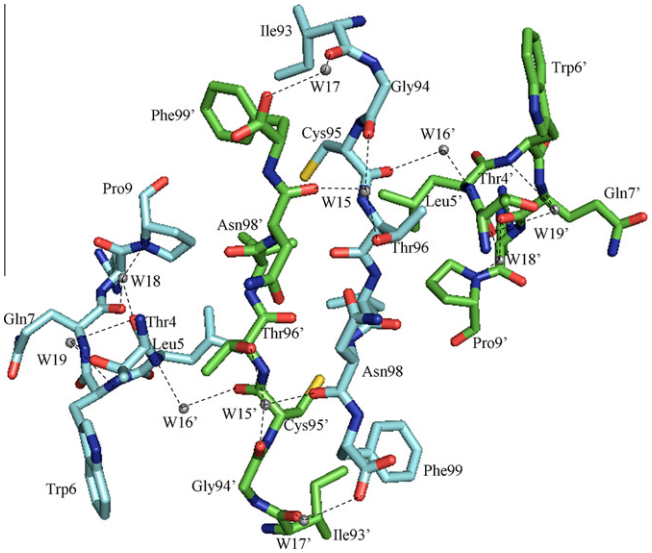

Fig. 3. The conserved crystallographic water molecules stabilizing the dimerization interface of MDR HIV-1 protease substrate complexes. The MDR HIV-1 protease monomers are shown in stick model and the crystallographic water molecules are shown in grey spheres. The black dash line represents the interaction between the crystallographic water molecules and the MDR HIV-1 protease.

4. Discussion

4.1. Missing conserved flap-ligand bridging water in MDR complex that is critical in substrate cleavage

Wild type HIV-1 protease studies show a bridging water molecule, critical in substrate cleavage, connecting the protease flaps Ile 50 N/Ile 50' N with P2 O/P1′ O. In the present study, this water molecule is missing in all the nine MDR complexes. Moreover, in eight out of nine MDR complexes, there is one water molecule located between the flaps, but can not reach either Ile 50 N/Ile 50' N or P2 O/P1′ O, due to the wide opening of the flaps. Nevertheless, this water molecule can still interact indirectly with the MDR protease flaps, suggesting its stabilization role to the flap area.

It is possible that the cleavage mechanism of the MDR complexes is different from the one of WT complexes, with the absence of the flap-ligand bridging water. This may explain why MDR HIV-1 protease is resistant to current FDA approved HIV-1 protease inhibitors. As a result, new HIV-1 protease inhibitors are required, that do not need the flap-ligand bridging water to mimic the transitional state.

4.2. Reduced hydrogen bonds between the ligand and protease backbone imply drug resistance

An extensive hydrogen-bonding interaction between the inhibitors and the HIV-1 protease backbone is believed to be one of the keys to overcome drug resistance [35,36]. However, our crystal structure analysis demonstrates significantly reduced hydrogen bonds between the substrate peptide and the MDR HIV-1 protease backbone atom, compared with those found in WT protease substrate peptide complexes. Among the nine MDR HIV-1 protease substrate peptide complexes, there are four conserved and two non-conserved hydrogen bonds between the ligand and protease backbone atoms, while eight highly conserved hydrogen bonds are found in the WT counterparts. This finding may imply the importance to restore the extensive protease backbone hydrogen bonding in order to overcome the drug resistance brought by the mutations. In addition, the loss of protease backbone hydrogen

bonds may explain the significant drug resistance of MDR HIV-1 protease against peptidomimetic-based HIV-1 protease. This conjecture is further supported by fact that Darunavir, a less peptidomimetic-based HIV-1 protease, shows relatively less drug resistance to MDR HIV-1 protease [13].

Acknowledgments

This research was supported by the National Institutes of Health grant AI65294 and a grant from the American Foundation for AIDS Research (106457-34-RGGN).

References

[1] J.A. Bartlett, R. DeMasi, J. Quinn, C. Moxham, F. Rousseau, Overview of the effectiveness of triple combination therapy in antiretroviral-naive HIV-1 infected adults, AIDS 15 (2001) 1369–1377.
[2] F.J. Palella Jr., K.M. Delaney, A.C. Moorman, M.O. Loveless, J. Fuhrer, G.A. Satten, D.J. Aschman, S.D. Holmberg, Declining morbidity and mortality among patients with advanced human immunodeficiency virus infection. HIV Outpatient Study Investigators, N. Engl. J. Med. 338 (1998) 853–860.
[3] V. Natarajan, M. Bosche, J.A. Metcalf, D.J. Ward, H.C. Lane, J.A. Kovacs, HIV-1 replication in patients with undetectable plasma virus receiving HAART. Highly active antiretroviral therapy, Lancet 353 (1999) 119–120.
[4] R.T. Schooley, J.W. Mellors, No cure yet for HIV-1, but therapeutic research presses on, J. Infect. Dis. 195 (2007) 770–772.
[5] A.J. McMichael, T. Hanke, HIV vaccines 1983–2003, Nat. Med. 9 (2003) 874–880.
[6] Y. Takeuchi, T. Nagumo, H. Hoshino, Low fidelity of cell-free DNA synthesis by reverse transcriptase of human immunodeficiency virus, J. Virol. 62 (1988) 3900–3902.
[7] J.D. Roberts, B.D. Preston, L.A. Johnston, A. Soni, L.A. Loeb, T.A. Kunkel, Fidelity of two retroviral reverse transcriptases during DNA-dependent DNA synthesis in vitro, Mol. Cell. Biol. 9 (1989) 469–476.
[8] J. Weber, F. Grosse, Fidelity of human immunodeficiency virus type I reverse transcriptase in copying natural DNA, Nucleic Acids Res. 17 (1989) 1379–1393.
[9] F. Clavel, A.J. Hance, HIV drug resistance, N. Engl. J. Med. 350 (2004) 1023–1035.
[10] C. Sukasem, V. Churdboonchart, W. Sukeepaisarncharoen, W. Piroj, T. Inwisai, M. Tiensuwan, W. Chantratita, Genotypic resistance profiles in antiretroviral-naive HIV-1 infections before and after initiation of first-line HAART: impact of polymorphism on resistance to therapy, Int. J. Antimicrob. Agents 31 (2008) 277–281.
[11] R.M. Gulick, J.W. Mellors, D. Havlir, J.J. Eron, A. Meibohm, J.H. Condra, F.T. Valentine, D. McMahon, C. Gonzalez, L. Jonas, E.A. Emini, J.A. Chodakewitz, R. Isaacs, D.D. Richman, 3-year suppression of HIV viremia with indinavir, zidovudine, and lamivudine, Ann. Intern. Med. 133 (2000) 35–39.
[12] J.H. Condra, W.A. Schleif, O.M. Blahy, L.J. Gabryelski, D.J. Graham, J.C. Quintero, A. Rhodes, H.L. Robbins, E. Roth, M. Shivaprakash, et al., *In vivo* emergence of HIV-1 variants resistant to multiple protease inhibitors, Nature 374 (1995) 569–571.
[13] Y. Wang, Z. Liu, J.S. Brunzelle, I.A. Kovari, T.G. Dewdney, S.J. Reiter, L.C. Kovari, The higher barrier of darunavir and tipranavir resistance for HIV-1 protease, Biochem. Biophys. Res. Commun. 412 (2011) 737–742.
[14] B.G. Turner, M.F. Summers, Structural biology of HIV, J. Mol. Biol. 285 (1999) 1–32.
[15] F. Liu, P.I. Boross, Y.F. Wang, J. Tozser, J.M. Louis, R.W. Harrison, I.T. Weber, Kinetic, stability, and structural changes in high-resolution crystal structures of HIV-1 protease with drug-resistant mutations L24I, I50V, and G73S, J. Mol. Biol. 354 (2005) 789–800.
[16] Y. Tie, P.I. Boross, Y.F. Wang, L. Gaddis, F. Liu, X. Chen, J. Tozser, R.W. Harrison, I.T. Weber, Molecular basis for substrate recognition and drug resistance from 1.1 to 1.6 angstroms resolution crystal structures of HIV-1 protease mutants with substrate analogs, FEBS J. 272 (2005) 5265–5277.
[17] B. Mahalingam, J.M. Louis, J. Hung, R.W. Harrison, I.T. Weber, Structural implications of drug-resistant mutants of HIV-1 protease: high-resolution crystal structures of the mutant protease/substrate analogue complexes, Proteins 43 (2001) 455–464.
[18] B. Mahalingam, P. Boross, Y.F. Wang, J.M. Louis, C.C. Fischer, J. Tozser, R.W. Harrison, I.T. Weber, Combining mutations in HIV-1 protease to understand mechanisms of resistance, Proteins 48 (2002) 107–116.
[19] M. Prabu-Jeyabalan, E. Nalivaika, C.A. Schiffer, How does a symmetric dimer recognize an asymmetric substrate? A substrate complex of HIV-1 protease, J. Mol. Biol. 301 (2000) 1207–1220.
[20] M. Prabu-Jeyabalan, E. Nalivaika, C.A. Schiffer, Substrate shape determines specificity of recognition for HIV-1 protease: analysis of crystal structures of six substrate complexes, Structure 10 (2002) 369–381.
[21] R.S. Yedidi, G. Proteasa, J.L. Martinez, J.F. Vickrey, P.D. Martin, Z. Wawrzak, Z. Liu, I.A. Kovari, L.C. Kovari, Contribution of the 80s loop of HIV-1 protease to the multidrug-resistance mechanism: crystallographic study of MDR769 HIV-1 protease variants, Acta Crystallogr. D: Biol. Crystallogr. 67 (2011) 524–532.
[22] P. Martin, J.F. Vickrey, G. Proteasa, Y.L. Jimenez, Z. Wawrzak, M.A. Winters, T.C. Merigan, L.C. Kovari, "Wide-open" 1.3 A structure of a multidrug-resistant HIV-1 protease as a drug target, Structure 13 (2005) 1887–1895.
[23] R.S. Yedidi, Z. Liu, Y. Wang, J.S. Brunzelle, I.A. Kovari, P.M. Woster, L.C. Kovari, D. Gupta, Crystal structures of multidrug-resistant HIV-1 protease in complex with two potent anti-malarial compounds, Biochem. Biophys. Res. Commun. 421 (2012) 413–417.
[24] Z. Liu, Y. Wang, J. Brunzelle, I.A. Kovari, L.C. Kovari, Nine crystal structures determine the substrate envelope of the MDR HIV-1 protease, Protein J. 30 (2011) 173–183.
[25] The CCP4 suite: programs for protein crystallography, Acta Crystallogr. D: Biol. Crystallogr. 50 (1994) 760–763.
[26] B.C. Logsdon, J.F. Vickrey, P. Martin, G. Proteasa, J.I. Koepke, S.R. Terlecky, Z. Wawrzak, M.A. Winters, T.C. Merigan, L.C. Kovari, Crystal structures of a multidrug-resistant human immunodeficiency virus type 1 protease reveal an expanded active-site cavity, J. Virol. 78 (2004) 3123–3132.
[27] G.N. Murshudov, A.A. Vagin, E.J. Dodson, Refinement of macromolecular structures by the maximum-likelihood method, Acta Crystallogr. D: Biol. Crystallogr. 53 (1997) 240–255.
[28] A.A. Vagin, R.A. Steiner, A.A. Lebedev, L. Potterton, S. McNicholas, F. Long, G.N. Murshudov, REFMAC5 dictionary: organization of prior chemical knowledge and guidelines for its use, Acta Crystallogr. D: Biol. Crystallogr. 60 (2004) 2184–2195.
[29] P. Emsley, K. Cowtan, Coot: model-building tools for molecular graphics, Acta Crystallogr. D: Biol. Crystallogr. 60 (2004) 2126–2132.
[30] V.S. Lamzin, K.S. Wilson, Automated refinement of protein models, Acta Crystallogr. D: Biol. Crystallogr. 49 (1993) 129–147.
[31] A.A. Vaguine, J. Richelle, S.J. Wodak, SFCHECK: a unified set of procedures for evaluating the quality of macromolecular structure-factor data and their agreement with the atomic model, Acta Crystallogr. D: Biol. Crystallogr. 55 (1999) 191–205.
[32] E. Krissinel, K. Henrick, Inference of macromolecular assemblies from crystalline state, J. Mol. Biol. 372 (2007) 774–797.
[33] A.C. Wallace, R.A. Laskowski, J.M. Thornton, LIGPLOT: a program to generate schematic diagrams of protein-ligand interactions, Protein Eng. 8 (1995) 127–134.
[34] E. Krissinel, K. Henrick, Secondary-structure matching (SSM), a new tool for fast protein structure alignment in three dimensions, Acta Crystallogr. D: Biol. Crystallogr. 60 (2004) 2256–2268.
[35] A.K. Ghosh, B.D. Chapsal, I.T. Weber, H. Mitsuya, Design of HIV protease inhibitors targeting protein backbone: an effective strategy for combating drug resistance, Acc. Chem. Res. 41 (2008) 78–86.
[36] A.K. Ghosh, Z.L. Dawson, H. Mitsuya, Darunavir, a conceptually new HIV-1 protease inhibitor for the treatment of drug-resistant HIV, Bioorg. Med. Chem. 15 (2007) 7576–7580.