Structural Basis of Drug Resistant I50V/A71V HIV-1 Protease and Gag Substrate Coevolution

Kuan-Hung Lin, Aysegul Ozen , Nese Kurt Yilmaz , Celia A. Schiffer

Department of Biochemistry and Molecular Pharmacology, University of Massachusetts Medical School, Worcester, MA 01605

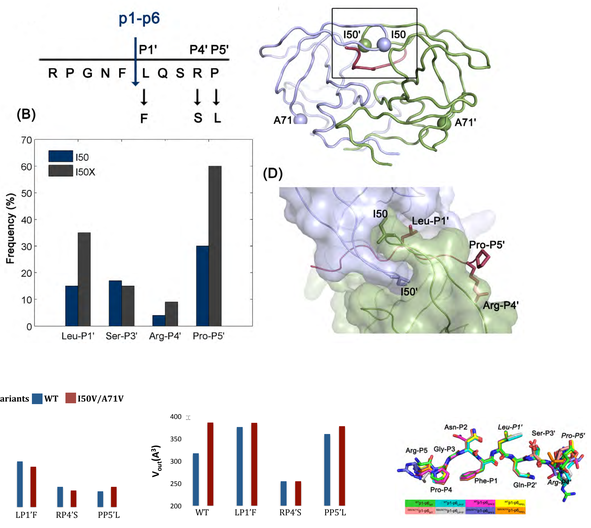
Celia.Schiffer@umassmed.edu

Background: Human immunodeficiency virus type-1 (HIV-1) protease is responsible for the processing of Gag and Gag-Pol viral polyproteins to release viral enzymes. As this process is essential for the maturation of HIV viral particles, the protease is an important target for anti-HIV therapy. Within major primary drug resistance mutations, mutation of residue 50 from Ile to Val is commonly observed in patients failing therapy with APV and DRV (two FDA approved protease inhibitors). Residue 50 is located at the flap tip of the flexible loop that controls the access of inhibitors and substrates to the protease active site. Substrate mutations Gag L449F, R452S and P453L within the p1-p6 cleavage site are associated with I50V protease mutation in patient sequences. Gag L449F mutation rescues the protease activity by 10 fold, while P453L, although located distal from the catalytic site, causes a 23-fold enhancement. However, the molecular basis for the selection advantage of these co-evolution mutations and how they enhance substrate recognition to confer drug resistance is not clear.

Method: By applying crystallography method and molecular dynamics simulation, we investigate the structural basis of I50V/A71V protease and p1-p6 substrate co-evolution.

Results: Based on crystal structures, substrates with mutated residues P1’ phenylalanine or P5’ leucine rescue protease activity by increased vdW contacts with co-evolved protease. I50V/A71V$_{RP4'S}$ complex structure has more hydrogen bond compared to I50V/A71V$_{WT}$, and more vdW contacts compared to WT$_{RP4'S}$. From the result of MD simulations, with mutations in either protease or substrate, the actives site are expanded (80-80 loop). Strikingly, co-evolution brings this distance back to the scale similar to WT$_{WT}$. The expanded protease active site may correlate with the decreased protease processing, while coevolution of both restores the active site dynamics and possibly the protease activity.

HIV-1 I50V/A71V protease and p1-p6 substrate mutations

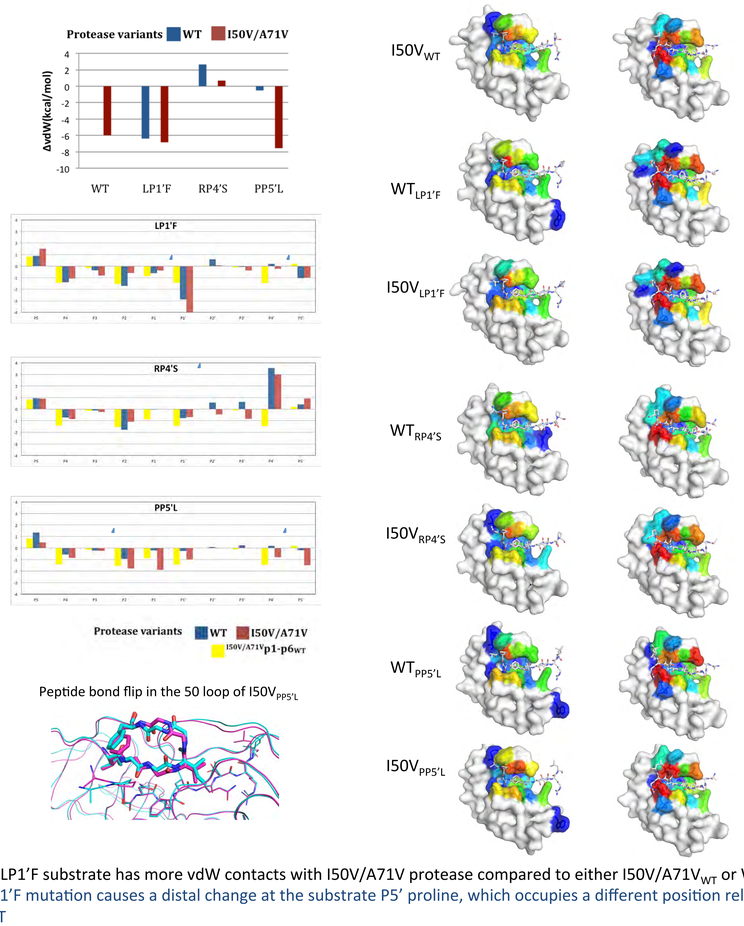


The overall backbone conformation of substrate–protease complexes is conserved in all mutant structures (RMSD of Ca atoms are within 0.48 Å). All the substrate residues from P3 to P3’ overlap well as well, and the substrate mutants maintain a comparable fit within the substrate envelope regardless whether the protease carries the I50V/A71V mutations or not

Crystallographic Statistics

	WT$_{WT}$	WT$_{LP1'F}$	WT$_{RP4'S}$	WT$_{PP5'L}$	I50V$_{WT}$	I50V$_{LP1'F}$	I50V$_{RP4'S}$	I50V$_{PP5'L}$
resolution (A°)	2	1.85	1.84	1.54	2.14	1.5	1.67	2
space group	P212121	P21	P212121	P212121	P21	P21	P21	P21
a (A°)	51.3	51.1	50.9	51.0	52.2	51.4	51.6	51.3
b (A°)	59.1	62.8	57.9	58.2	63.3	59.8	58.2	62.7
c (A°)	61.8	61.5	61.8	61.8	58.8	60.6	61.8	57.5
Rmerge (%)	6.7	7.3	10.4	4.6	3.3	5.6	4.7	7.6
completeness (%)	93.4	99.7	98.4	97.1	94.5	97.5	94.6	94
total no. of reflections	41786	113050	127633	195712	69773	220012	119749	64360
no. of unique reflections	12376	28931	16417	27160	20171	56731	40242	23065
no. of molecules	1	2	1	1	2	2	2	2
Rfree (%)	25.1	21.28	20.35	17.85	23.84	20.80	22.93	23.62
Rfactor (%)	20.3	16.3	16.00	15.34	18.35	17.18	17.85	17.06
rms in bond length (A°)	0.006	0.009	0.0092	0.0087	0.0097	0.0094	0.0091	0.0094
rms angle (deg)	1.3	1.423	1.500	1.347	1.462	1.385	1.436	1.461
no. of water	101	215	151	197	275	404	327	177

Alterations in van der Waals contacts



Peptide bond flip in the 50 loop of I50V$_{PP5'L}$

- The LP1’F substrate has more vdW contacts with I50V/A71V protease compared to either I50V/A71V$_{WT}$ or WT$_{LP1'F}$
  - LP1’F mutation causes a distal change at the substrate P5’ proline, which occupies a different position relative to WT
- The RP4’S substrate has more overall vdW contacts in the coevolved I50V/A71V$_{RP4'S}$ complex relative to WT$_{RP4'S}$ but less than the WT substrate in I50V/A71V$_{WT}$
  - mutation at P4’ residue actually affects the interactions at other positions, P3’ Ser and P2’ Gln has enhanced vdW contacts in I50V/A71V$_{RP4'S}$ compared to either I50V/A71V$_{WT}$ or WT$_{RP4'S}$
- PP5’L mutation increases the P5’ residue’s interactions with I50V/A71V protease as the leucine packs closer to the 50s loop of chain B
  - The peptide bond between Gly51 and Gly52 in the I50V/A71V$_{PP5'L}$ structure is flipped compared to the other structures, and this flipped peptide bond pushes the 50s loop toward the substrate

Mutated P4’ serine contributes one more hydrogen bond

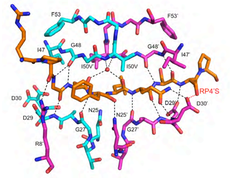
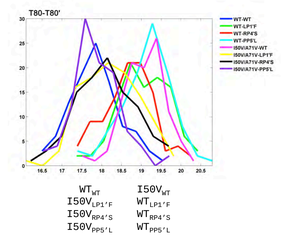


Table 2. Hydrogen bonds

protease	substrate	WT$_{WT}$	I50V$_{WT}$	I50V$_{LP1'F}$	WT$_{LP1'F}$	I50V$_{RP4'S}$	WT$_{RP4'S}$	I50V$_{PP5'L}$	WT$_{PP5'L}$
B Arg 8 NH2	Pro 2 O	3.01	3.01	2.72	2.73	2.94	2.97	2.89	2.94
A Gly 48 O	Gly 3 N	3.01	2.97	3.03	2.77	2.90	3.03	2.86	3.03
A Asp 29 N	Gly 3 O	2.79	2.97	2.65	2.86	2.96	2.93	2.82	2.88
A Gly 48 O	Asn 4 N	3.05	2.86	2.88	2.74	2.85	2.88	2.96	2.95
A Gly 27 O	Phe 5 N	2.93	2.90	2.81	2.88	2.90	2.80	2.78	2.88
B Asn 25 ND2	Phe 5 O	2.7	3.07/2.7	2.72	2.74	3.0/2.9	3.7/2.7	2.70	2.76
B Gly 27 O	Gln 7 N	2.95	2.94	2.97	3.07	2.91	2.83	2.95	2.98
B Asp 30 N	Gln 7 OE1	2.74	2.72	2.89	2.86	2.81	2.82	2.83	2.77
B Asp 30 OD2	Gln 7 NE2	2.95	2.73	2.79	3.02	2.81	3.6/2.8	2.98	2.88
B Asp 29 N	Gln 7 O	3.07	3.07	3.12	3.15	3.04	3.01	3.06	3.10
B Gly 48 O	Ser 8 N	3.00	2.98	3.03	3.09	2.81	3.00	3.04	3.02
B Gly 48 N	Ser 8 O	2.78	2.91	3.07	3.26	2.97	3.01	3.02	3.02
B Asp 29 OD2	Arg 9 N/Ser 9 N	3.0	2.95	2.85	2.98	2.96	3.06	2.84	2.94
B Asp 30 OD2	Arg 9 NE/Ser 9 OG	3.02				2.59	3.6/2.9		

The fluctuations of 80 loops (MD simulations)





WT$_{WT}$ I50V$_{WT}$
I50V$_{LP1'F}$ WT$_{LP1'F}$
I50V$_{RP4'S}$ WT$_{RP4'S}$
I50V$_{PP5'L}$ WT$_{PP5'L}$

In the WT$_{WT}$ structure, the distance between these two loops is around 17.5 Å, and with mutations in either protease or substrate, it expands to 19–19.5 Å. Strikingly, co-evolution brings this distance back to 17.5–18 Å, which is similar to the distance in WT$_{WT}$. The expanded protease active site may correlate with the decreased protease processing activity

Conclusions

- Coevolved complex structures display enhanced overall substrate interactions, due to either more vdW contacts or hydrogen bonds, compared to structures with mutation in either protease or substrate alone.
- The effects of protease-substrate co-varitation is not restricted to the mutation sites, it can cause the structural change of protease and substrate at non-mutated positions and increase the overall fitting of substrate in the protease active site.
- Through MD simulations, we show that the co-variation groups tend to maintain the WT$_{WT}$ scale of active site volume while single mutation groups have expand active site.

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