Concerted hydrolysis mechanism of HIV-1 natural substrate against subtypes B and C-SA PR: Insight through Molecular Dynamics and Hybrid QM/MM studies

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The HIV-1 PR cleavage sites in the Gag and Gag-Pol polyproteins.

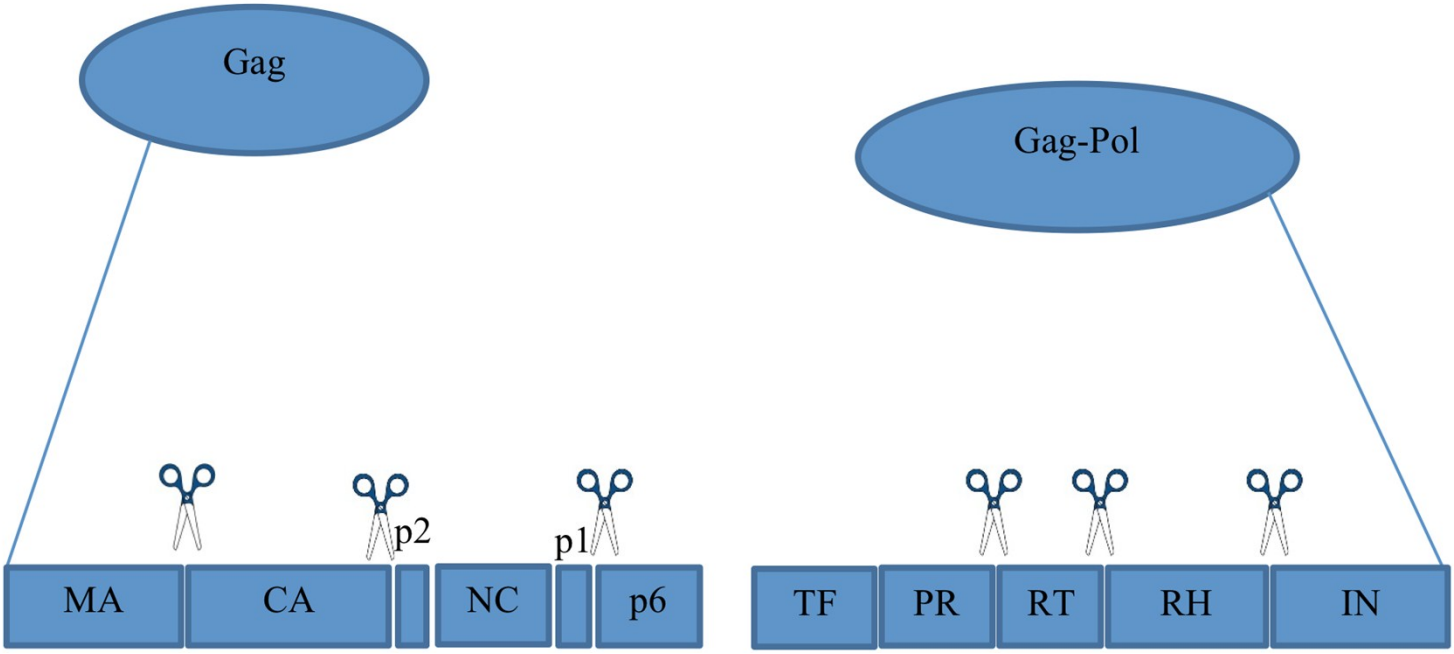

Figure S1. Schematic representation of the Gag and Gag-Pol polyproteins.

We determined (a) the standard deviation for these calculations and (b) explored whether the MD method to find starting structures for these calculations will give rise to potential conformational effects of the HIV-1 PR—substrate complexes. In order to achieve this, we performed a triplicate MD run for the PR-RT and TF-PR complexes, since these two substrates gave a reversed order for the theoretical activation energies, compared to the experimental results. We employed a random seed to create a new starting MD simulation with different atomic velocities, creating a new starting MD run with different atomic coordinates as well as different atomic velocities and changing the simulation box.[1] This was followed by a ONIOM (QM/MM) geometry optimization on the three different starting structures from the MD calculation (Table S1). The optimized ONIOM (QM/MM) TS structures for PR-RT and TF-PR enzyme—substrate complexes taken from the triplicate MD runs as well as the lowest substrate conformation taken from the restrained MD (described earlier) exhibit a similar geometry overall. Note that the standard deviation for both substrates PR-RT and TF-PR were as high as 1.0 and 0.7 kcal mol^{-1} respectively. The differences in distances are small (Figure S2) with an average RMSD of 0.82 Å, this suggests that

the reversed orders for the activation energies is not a result of different poses for the starting structures. It also gives assurance that the method to create starting structures produces consistent minimum conformations for the substrates.

Table S1. The relative thermodynamics of the three ONIOM (QM/MM) optimized MD run of PR-RT and TF-PR enzyme—substrate complexes as well as the lowest conformation using ONIOM [B3LYP/6-31++G(d,p)].

HIV-1 PR B—PR-RT	**ΔG‡**	**Average value**	**Standard deviation**
1. ONIOM after lowest conformation	14.5		
2. ONIOM after random seed in MD	14.5		
3. ONIOM after creating a different atomic coordinates and velocities in MD	15.7	15.3	1.0
4. ONIOM after changing the size of simulation box in MD	16.6		
HIV-1 PR B—TF-PR			
1. ONIOM after lowest conformation	16.5		
2. ONIOM after random seed in MD	16.6		
3. ONIOM after creating a different atomic coordinates and velocities in MD	17.5	17.1	0.7
4. ONIOM after changing the size of simulation box in MD	17.9		

Values are reported in kcal mol^{-1}, ΔG‡ =Activation free energy

The ONIOM calculation after changing the size of the simulation box[1, 2] from 10 to 12 Å should not have a drastic effect on the interaction between the natural substrate and the active sites. As mentioned earlier the average RMSD is less than 1 Å which shows good pose for the natural substrate.[3, 4]

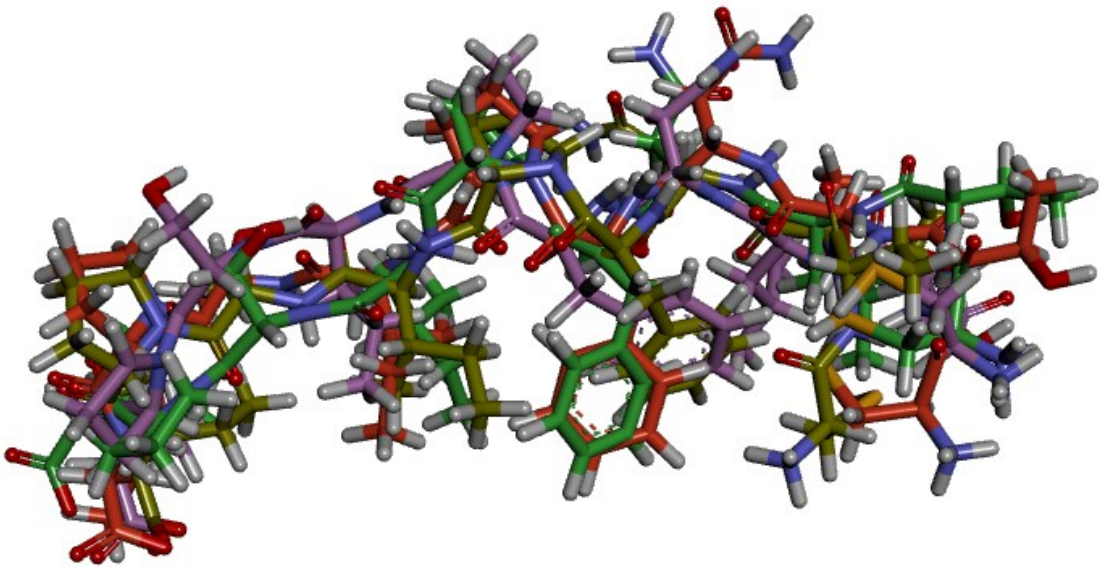

Figure S2. Superimposed PR-RT natural substrate (average RMSD of 0.82 Å) taken from triplicate MD runs showing movement and flexibility at the subtype B HIV-1 PR active site.

However, the structural characteristic of the complexes may have been altered as a result of the increase in the water residues resulting from the change in the simulation box.[5] Therefore, a virtual inspection and analysis was performed for the PR-RT and TF-PR for cases 1 and 4 (Table S1) using LigPlot[6] (see Figure S3a and S3b). As can be seen more hydrogen bond intermolecular interactions was observed for case 1 (Table S1), while case 4, after changing the size of simulation box in MD complex shows fewer hydrogen bond intermolecular interactions between the substrate and active sites. This explains why case 1 has a much lower activation energy compared to case 4 for both natural substrates Figure S3a and S3b.

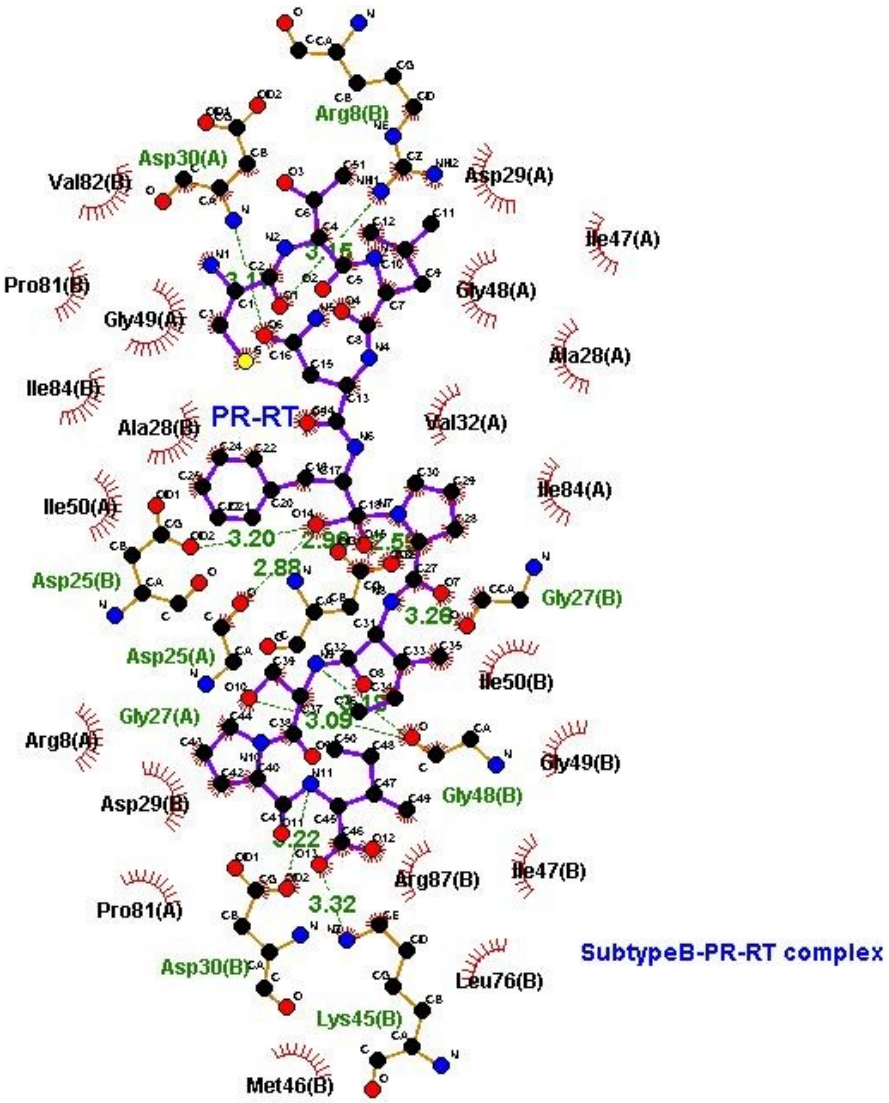

Figure S3a. Hydrogen bond interactions plot of HIV subtype B PR complexed with PR-RT natural substrate. These plots were created after ONIOM optimization of the lowest conformation (case 1) using Ligplot[6].

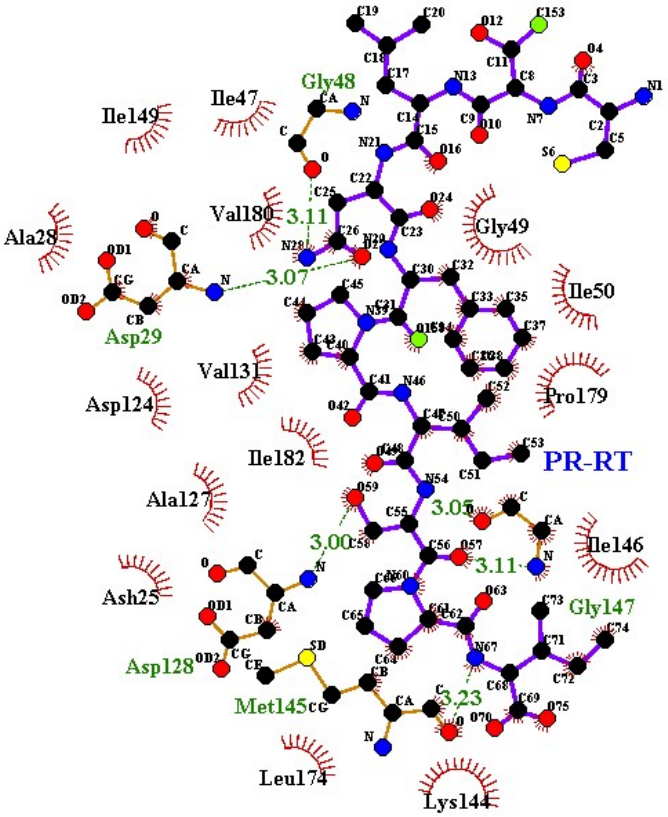

Figure S3b. Hydrogen bond interactions plot of HIV subtype B PR complexed with PR-RT natural substrate. These plots were created after ONIOM optimization of the changed simulation box to 12 Å using Ligplot[6].

Appendix: The 3D structures of all the enzyme—substrate complexes considered.

3D structures of the enzyme substrate complexes.zip

References

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