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ORIGINAL ARTICLE

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Computational Study of the Effect of M204V Mutation on Lamivudine Resistance in Hepatitis B Virus

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Keywords

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ABSTRACT • Hepatitis B virus DNA polymerase is an important antiviral drug target in treatment of the Hepatitis B virus infection. Lamivudine, a widely used antiviral drug, plays an important role in the treatment of infection by inhibiting Hepatitis B virus DNA polymerase. There is no experimental Hepatitis B virus DNA polymerase structure in the data banks; Therefore, in this study we developed a Hepatitis B virus DNA polymerase model by homology modeling. Human immunodeficiency virus was used as a template to create the homology model of Hepatitis B virus DNA polymerase. Tyrosine (Y), methionine (M), aspartic acid (D), and aspartic acid (D) motifs in the catalytic site are identical in both structures. However, the similarity rate between their sequences is 30%. After the homology model was developed, the structure was equilibrated with a 50 ns molecular dynamics simulation. Using molecular docking technique, lamivudine binding to hepatitis B virus DNA polymerase was investigated by calculating binding free energy. As a second binding study, the methionine (M) found in the Y-M-D-D motif was mutated to valine (V) amino acid and the binding energy was calculated again. There is a difference of about 2 kcal / mol between the two binding energies. This difference suggests that this mutation in the catalytic site produces a resistance to lamivudine.

INTRODUCTION

Hepatitis B virus infection (HBV) is a common health problem that causes many deaths worldwide (1,2). Chronic infection causes cirrhosis and liver cancer (3). Antiviral drugs are generally used in the treatment of chronic HBV infection. Lamivudine, has a good efficiency on HBV DNA polymerase (HDP) inhibition (4,5). However, resistance to lamivudine has become a major problem over time. This has opened the way for investigating the causes of resistance. Lamivudine resistance is associated with mutations in the highly conserved tyr (Y), met (M), asp (D), asp (D) catalytic motifs in reverse transcriptase (RT) domain of HDP (6-8). It is known that M204V mutation in the YMDD motif play a key role in resistance to Lamivudine (9). In recent years, advances in bioinformatics have made way for computational techniques. With increasing experimental 3D structures in databanks, it has become widespread to investigate the functional properties of biological

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structures using techniques such as molecular dynamics (MD) simulations. Homology modeling is a useful technique in the absence of experimental 3D structure (10). There are studies about constructing a homology model for HDP structure (8, 11-13). All of these models used the human immunodeficiency virus (HIV) (PDB ID: 1RTD) structure as a template. HIV and HDP structures have the same catalytic YMDD motif, indicating good homology between them. In this study, we have constructed a homology model of HDP using HIV structure as a template. Then, MD simulations were carried out for equilibration. After a 50 ns MD simulation, Lamivudine binding is investigated between wild type and mutant HDP structures with Docking process. Our main purpose in this study is to give an overview of how a point mutation will affect the binding energy.

MATERIALS and METHODS

Homology Modeling

Homology Modeling is the process of determining an unknown 3D structure based on a known experimental structure. Finding a suitable experimental structure, also called a template, is the first step in this process. In this study, since the 3D structure of HDP is not known, the structure has been determined by homology modeling. The 3D structure of HIV-1 reverse transcriptase (PDB ID: 1RTD) (14), which was also mentioned as a good template in previous studies (8, 11-13), is used as a template. The main reason why the HIV-1 structure is regarded as a good template is the high similarity of the catalytic site in both structures. After a good template is found, the second step of the homology modeling is the sequence alignment. Alignment is a process of comparing sequences of two or more structures. After the alignment process, homology models can be generated through some softwares. Modeller 9.20 (15) is used in this study, as a homology modeling tool.

Molecular Dynamics and Docking

MD simulations are performed using NAMD 2.11 (16) package with CHARMM36 force field (17). Simulation system is designed for NPT ensemble using periodic boundary conditions. Pressure is maintained at 1 atm and Temperature is maintained at 300K using Langevin dynamics with Langevin damping coefficients of 5 s^{-1} (18). Particle-mesh Ewald algorithm is used for electrostatic interactions. Within a distance of 10-12 Å , Lennard-Jones interactions are switched off and a time step of 2 fs is used for all simulations. In order to determine the binding energy of Lamivudine structure, Docking processes are performed using AutoDock Vina (19). Structure of Lamivudine is collected from PubChem (20) and that structure is used as an input for CGenFF program (21) to obtain topology and parameter files. Topology file and psfgen package of VMD software (22) are used to obtain a 3D structure of Lamivudine.

RESULTS

Homology Modeling of HDP

Sequence alignment between HDP and HIV (1rtd.pdb) structures was carried out using EMBOSS-Needle (23) pairwise sequence alignment tool shown in Figure 1. There is a 30% similarity

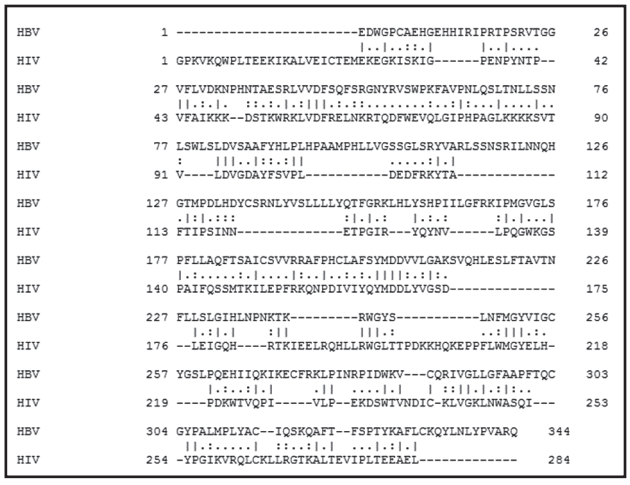

Figure 1 Sequence alignment

between sequences. As shown in the figure, the catalytic YMDD motif is conserved. In the alignment, single point shows different aminoacids, colons represent similar aminoacids and rods are used to show identical aminoacids between two sequences. After sequence alignment was completed, Modeller 9.20 was used for homology modeling. This software detects sequence differences and constructs the unknown structure by mutating from the template. After completing the 3D structure, molecular dynamics simulation is ready to be performed.

Molecular Dynamics Simulation of HDP

The 3D structure of HDP is solvated in a 40x40x40 Å water box. System is neutralized with a 150 nM NaCl concentration. There are 54437 atoms in the system. A relaxation process is applied at the beginning of the simulation. Simulation was started by applying a 10 kcal/mol constraint on backbone and sidechain atoms. The applied constraints were reduced for 5 ns and completely removed after 5 ns. RMSD behavior of the backbone atoms is shown in Figure 2. As shown in the graph, there are jumps during the first 5 ns. These jumps belong to the point at which the constraints are reduced and the largest jump appear at the beginning of the 5th ns when the constraints are completely removed. After the 5th ns, RMSD behavior continues in the plateau region. That means fluctuation of backbone atoms has been minimized. System is equilibrated with a 50 ns molecular dynamics simulation.

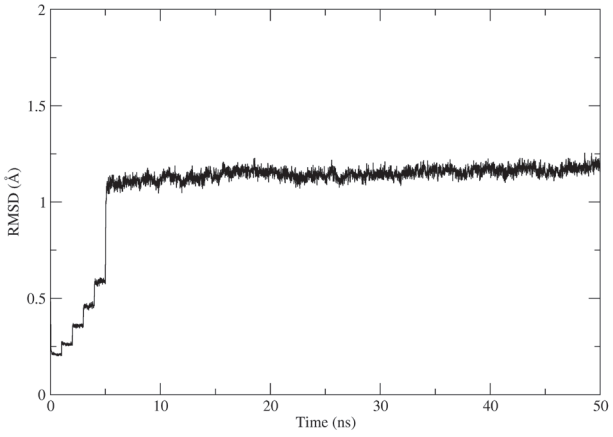

Figure 2 RMSD behaviour of backbone atoms.

Binding Free Energy Calculations

Docking is used to observe the effect of mutations on lamivudine resistance. Firstly, lamivudine binding to wild type HDP structure is investigated. The HDP structure is chosen as a rigid macromolecule in all docking studies. In default settings on AutoDock Vina, the macromolecule is always rigid and ligand is flexible. In this study, the catalytic YMDD motif is also set to be flexible. The main reason behind this is to count the effect of fluctuation in the catalytic region to the binding free energy calculation. In Figure 3, there is a snapshot obtained from the docking study. Green rods represent the YMDD motif and lamivudine is seen with spherical representation. This view belongs to the docking conformations of wild type HDP structure. After completing wild type docking studies, the M204V mutation is performed with the mutate residue function of the VMD software. After this process, the docking studies are repeated for mutant structure with same protocol and compared with the wild type studies. Table 1, shows binding free energy scores obtained from docking.

DISCUSSION

The binding energy can be defined as the energy needed to separate the bound structures. Lamivudine binding to wild type HDP has more negative binding free energy than the mutant one. It shows lamivudine binds weaker to HDP with the M204V mutation. The structure has gained resistance to Lamivudine with that mutation. As illustrated in Table 2, Muralidharan et al. reported 1.9 kcal/mol average binding free energy difference between native type HDP structure and M204I mutated structure (24). Muralidharan et al. also depicted that there is a 2 kcal/mol average binding free energy difference between native type HDP structure and M204V mutated structure which is shown in Table 3 (25). These results and the binding free energies obtained in this study shown in Table 1 are in correlation with each other.

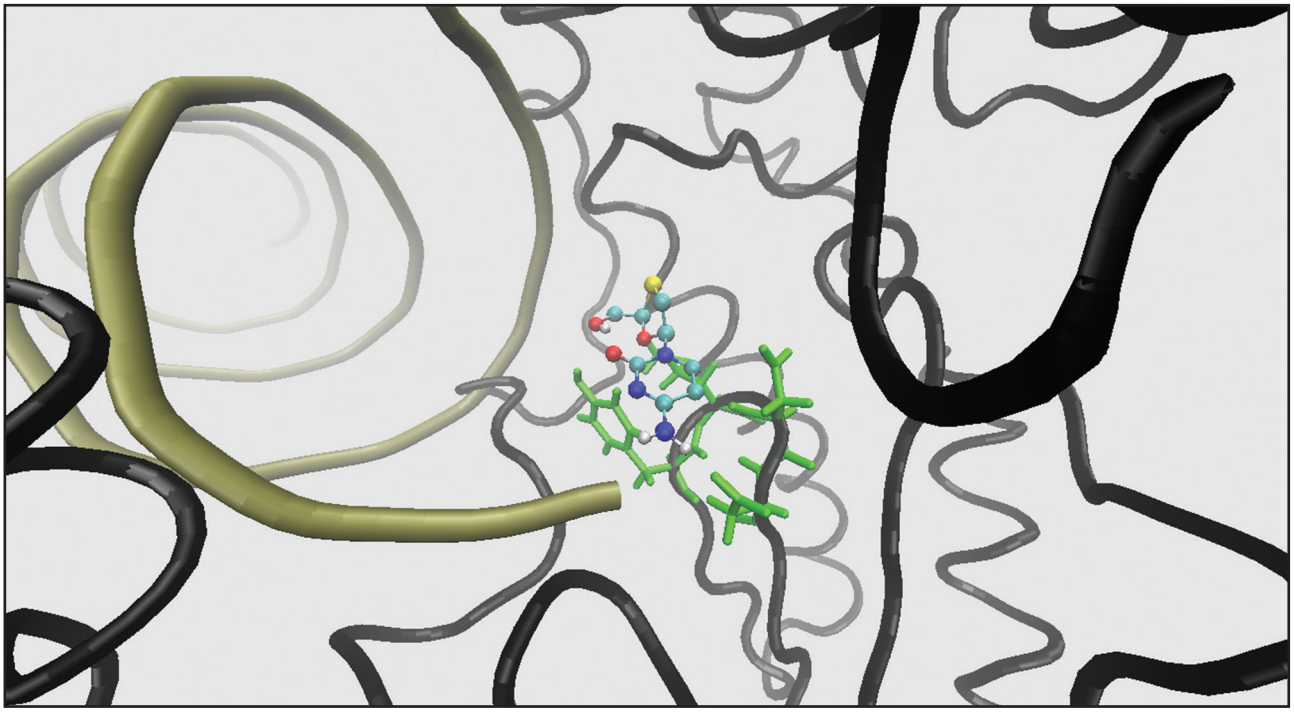

Figure 3 Lamivudine binding to hepatitis B virus DNA polymerase structure.

Table 1 Lamivudine binding free energies on wild type and mutant hepatitis B virus DNA polymerase structures.

Structure	Conformation 1 (kcal/mol)	Conformation 2 (kcal/mol)	Conformation 3 (kcal/mol)
Wild type HDP	-8.51	-8.4	-8.32
M204V mutation on HDP	-6.7	-6.5	-6.22

Table 2 Docking results of lamivudine binding to HDP structure [25].

Complex type	Docking 1 (kcal/mol)	Docking 2 (kcal/mol)	Docking 3 (kcal/mol)	Average binding free energy (kcal/mol)
Native type HDP	-8.23	-8.45	-8.27	-8.31
M204I mutation on HDP	-6.44	-6.47	-6.32	-6.41

Table 3 Docking results of lamivudine binding to HDP structure [25].

Complex type	Docking 1 (kcal/mol)	Docking 2 (kcal/mol)	Docking 3 (kcal/mol)	Average (kcal/mol)
Native type HDP	-8.90	-8.85	-8.70	-8.81
M204V mutation on HDP	-6.54	-6.86	-6.43	-6.61