Source of Oseltamivir Resistance Due to R152K Mutation of Influenza B Virus Neuraminidase: Molecular Modeling

J. Tengrang, T. Rungrotmongkol, S. Hannongbua

Abstract—Every 2-3 years the influenza B virus serves epidemics. Neuraminidase (NA) is an important target for influenza drug design. Although, oseltamivir, an oral neuraminidase drug, has been shown good inhibitory efficiency against wild-type of influenza B virus, the lower susceptibility to the R152K mutation has been reported. Better understanding of oseltamivir efficiency and resistance toward the influenza B NA wild-type and R152K mutant, respectively, could be useful for rational drug design. Here, two complex systems of wild-type and R152K NAs with oseltamivir bound were studied using molecular dynamics (MD) simulations. Based on 5-ns MD simulation, the loss of notable hydrogen bond and decrease in per-residue decomposition energy from the mutated residue K152 contributed to drug compared to those of R152 in wild-type were found to be a primary source of high-level of oseltamivir resistance due to the R152K mutation.

Keywords—Influenza B neuraminidase, Molecular dynamics simulation, Oseltamivir resistance, R152K mutant

I. INTRODUCTION

THE influenza virus is classified into three types based on antigens (A, B and C). Only types A and B can infect humans and thus they are of great epidemiological interest [1]. In every 2-3 years, influenza B virus always causes the seasonal flu outbreak with high morbidity, especially for children and adults living in closed environments [2]-[3].

Influenza viruses are the developed single-stranded RNA viruses. Both influenza A and B viruses consist of 8 separate segment genomes enclosed by a capsid protein. These segment genomes are used to build the functional proteins: polymerase B2 protein (PB2), polymerase B1 protein (PB1), polymerase A protein (PA), haemagglutinin (HA), nucleocapsid protein (NP), neuraminidase (NA), matrix protein (M: M1 and M2) and non-structural protein (NS) [4].

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The HA and NA glycoproteins appear on the surface membrane of the virus, and are both able to recognize the terminal sialic acid. HA binds to sialic acid via the carbohydrate side-chains of cell-surface glycoproteins and glycolipids. Following virus replication, the receptor destroying enzyme, NA, removes its substrate from the infected cell in order to infect new cells [5]. Up to date, there are two groups of anti-influenza agents: M2 inhibitors and NA inhibitors (NAIs). The M2 inhibitors amantadine and rimantadine are ineffective against influenza B virus, but the NAIs zanamivir and oseltamivir have been shown to be effective against the influenza B strain [3]. An oral drug oseltamivir is often used and stockpiled rather than zanamivir, which is administered by inhalation, resulted in the high amount of oseltamivir treatment in worldwide. Although oseltamivir has shown its inhibitory efficacy against viral influenza, numerous studies have been implemented to determine the rate of oseltamivir resistant isolates [6]-[9]. Some previous studies show that the drug resistance is directly associated with mutations in NA [10]. The emergence of oseltamivir resistance in influenza B virus variants with the R152K substitution in NA gene has been isolated [7]-[8], as summarized in Table I.

TABLE I
SUSCEPTIBILITY OF VIRAL INFLUENZA B NA STRAINS TO OSELTAMIVIR

R152K mutant	Method	IC_{50}, nM (wild-type / R152K)	Fold resistance[a]
B/Memphis/1996[7]	*in vivo*	4.0 / 400	100
B/Brec[8]	reverse genetic	1.8 / 462	256

[a] Fold resistance is the ratio of IC_{50} in the R152K and wild-type strains

Information on the drug resistant mechanism would be useful for rotational drug design to combat the influenza B virus. Molecular dynamics (MD) simulation is an approach used to study and understand the mechanisms of drug inhibition and resistance. In the present work, MD simulation was applied to demonstrate how oseltamivir loses its inhibitory efficiency against the viral influenza B R152K NA in comparison to the wild-type.

II. MATERIAL AND METHODS

Nowadays, there are three available crystal structures of influenza B virus NA strains, B/Beijing/1/87 [11], B/Lee [12] and B/Perth/211/2001 [13]. The crystal structure of wild-type B/Beijing NA with sialic acid bound obtained from Protein

Data Bank (PDB) code 1NSC [11], was chosen to use as the starting structure for the wild-type and R152K systems. The complex structure between wild-type NA and oseltamivir was constructed by replacing the sialic acid with the oseltamivir structure taken from PDB code 2HU4, where the crystallographic calcium ions and water molecules were kept. Then, to construct the R152K–oseltamivir complex, the residue 152 of the wild-type complex was singly mutated from arginine to lysine using the Leap module in AMBER10. The protonation states of the ionizable residues (K, R, D, E and H) were assigned according to the PROPKA prediction and manual verification [14].

All system preparations and atomistic MD simulations were carried out using the AMBER10 software package [15]. All minimizations and MD simulations were performed using the SANDER module implemented in AMBER. After adding hydrogen atoms, all hydrogen atoms of protein-ligand complexes were only minimized to release the bad contact. A TIP3P water box [16], with a minimum distance of 13 Å from protein surface to the edge of simulation box, was used to solvate the system. The system neutralization was treated by counter ions using the LEaP module. The water molecules were minimized by fixing the protein-ligand complex. Consequently, the whole system was minimized with 1,000 steps of steepest descent and 2,000 steps of conjugate gradient. A nonbonded interaction was cut-off at 12 Å [17]. The system was then heated to 310 K for 100 ps and equilibrated at this temperature for another 100 ps. Finally, the simulation was performed at 310 K with the NPT ensemble for 5 ns. The global root mean square deviation (RMSD) relative to the starting structure was monitored to verify the stability of simulated system. After the complex reached equilibrium, the drug-target interactions were analyzed using the ptraj and MM/PBSA modules of AMBER.

III. RESULTS AND DISCUSSION

The stability of all systems was accessed by considering the plot of root mean square displacement (RMSD) of MD structures versus simulation time in respect with the initial structure (Fig. 1). From 5 ns of MD simulations, all systems were found to reach to equilibrium at 3 ns, and thus the snapshots extracted from the last 2ns were used for further analysis.
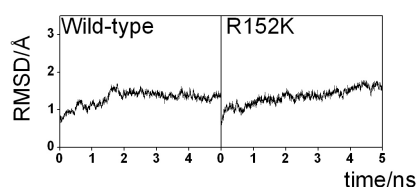

Fig. 1 RMSD Plot of protein backbone atoms versus simulation time for the wild-type and R152K complexes

The mutation of NA residue may affect the oseltamivir binding at the active site as can be seen by the hydrogen bonds between oseltamivir and its binding residues. Based on a maximum distance of 3.5 Å between hydrogen donor (D) and acceptor (A) and with a maximum bond angle of 120 degree for D_H...A, the percentage of hydrogen bond occupations in the two systems studied was shown in Fig. 2. A hydrogen bond with ≥ 75% of occupation is determined as the strong binding interaction. Besides, the per-residue decomposition free energy calculation was calculated to determine the contribution from individual residues at both active and framework sites to oseltamivir as plotted in Fig. 3.
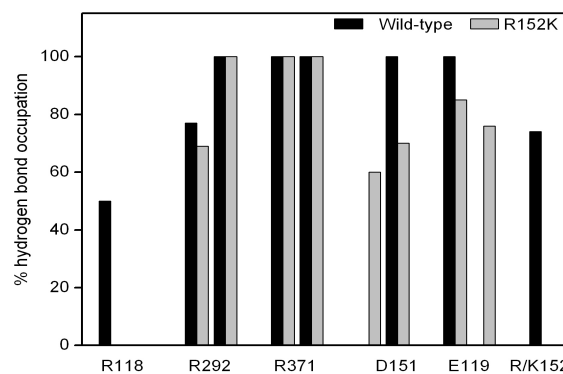

Fig. 2 Percentage occupation of hydrogen bonds formed between oseltamivir and the binding residues in the two NAs, wild-type and R152K mutant

In Fig. 2, among the 17 NA residues at the active and framework sites of wild-type NA, the R118, E119, D151, R152, R292 and R371 are the residues that attach to oseltamivir through strong hydrogen bond formations (≥ 75% occupation), except for R118 (50%). For the R152K mutated system, the loss of hydrogen bonds with R118 and the mutated residue K152 was observed. This result indicated that the mutation on the residue in the NA active site from the positively charged residue with long side chain, R152, to the shorter one, K152, significantly influences the oseltamivir binding strength.
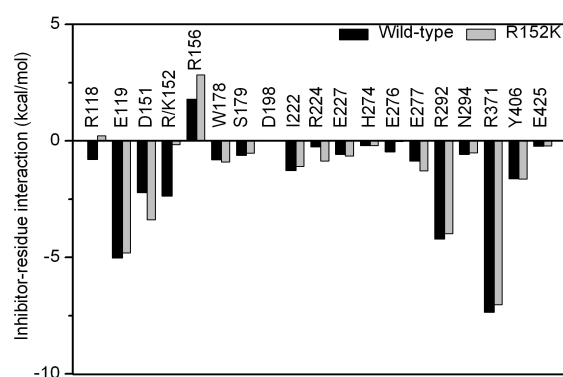

Fig. 3 Per-residue decomposition energy of residues at the catalytic and framework sites contributed to oseltamivir

By considering the plot of per-residue decomposition free energy for the wild-type system (Fig. 3), the major stabilization to oseltamivir was likely from all residues that directly formed hydrogen bond in Fig. 2. The additional

contribution was from the framework residues W178, S179, I222, R224, E227, E277 and Y406. Due to R152K mutation, decreased contribution of R118 and K152 were observed in good agreement with hydrogen bond results.

The oseltamivir binding affinity against influenza B NA strains for both wild-type and R152K mutant were predicted using MM/PBSA binding free energy calculation. As a result, the binding free energy ($\Delta G_{binding}$) and its components were summarized in Table II, where the experimental $\Delta G_{binding}$ converted from IC_{50} values [7]-[8] were also shown.

TABLE II
MM/PBSA CALCULATED BINDING FREE ENERGY AND ITS COMPONENTS
(KCAL/MOL) OF OSELTAMIVIR-NA COMPLEXS

COMPONENT ENERGIES (KCAL/MOL)	OSELTAMIVIR COMPLEXES WITH	
	WILD-TYPE	R152K
ΔE_{ele}	-171.9 ± 12.2	-171.1 ± 11.0
ΔE_{vdW}	-29.2 ± 3.0	-28.1 ± 3.3
ΔE_{MM}	-201.2 ± 11.0	-199.2 ± 9.5
$\Delta G_{sol}^{nonpolar}$	-5.0 ± 0.1	-5.0 ± 0.2
ΔG_{sol}^{polar}	172.9 ± 9.4	186.4 ± 8.8
$\Delta G_{binding}$	-33.7 ± 5.8	-25.5 ± 5.6
$\Delta G_{binding}^{[7]}$	-11.4	-8.7
$\Delta G_{binding}^{[8]}$	-11.9	-8.6

From Table II, the binding free energy of oseltamivir in wild-type and R152K systems were of -33.7 and -25.5 kcal/mol, respectively. This was as expected from the results of hydrogen bond and per-residue decomposition energy in which the oseltamivir showed the stronger binding to wild-type influenza B NA, while the increased binding free energy by 8.2 kcal/mol in R152K mutant system corresponded to the loss of oseltamivir-NA interaction. Taking into account, the mutation at NA active site, R152K, can directly affect the oseltamivir binding resulted in the high-level of oseltamivir resistance with 100-fold or 256-fold, as shown in Table I. The calculated binding free energy was found in good agreement with the experimental IC_{50} value.

IV. CONCLUSION

MD simulations were applied on the wild type and R152K mutant of viral influenza B NA in order to investigate the source of oseltamivir resistance. In comparison to the wild type, the loss of hydrogen bond interaction with the mutated residue K152 in the R152K system as well as the almost disappearance in per-residue decomposition energy at this residue contributed to oseltamivir were found to be a primary source of oseltamivir resistance with 100-fold or 256-fold relative to the wild-type. This is also supported by the MM/PBSA binding free energy, the oseltamivir is more favorable to the wild-type strain (-33.7 kcal/mol) than R152K mutant (-25.5 kcal/mol).

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