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Molecular Dynamics Study on Laninamivir Inhibiting Neuraminidases of H5N1 and pH1N1 Influenza a Viruses

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***Abstract*—**Viral influenza A subtypes H5N1 and pandemic H1N1 (pH1N1) have worldwide emerged and transmitted. The most common anti-influenza drug for treatment of both seasonal and pandemic influenza viruses is oseltamivir that nowadays becomes resistance to influenza neuraminidase. The novel long-acting drug, laninamivir, was discovered for treatment of the patients infected with influenza B and influenza A viruses. In the present study, laninamivir complexed with wild-type strain of both H5N1 and pH1N1 viruses were comparatively determined the structures and drug-target interactions by means of molecular dynamics (MD) simulations. The results show that the hydrogen bonding interactions formed between laninamivir and its binding residues are likely similar for the two systems. Additionally, the presence of intermolecular interactions from laninamivir to the residues in the binding pocket is established through their side chains in accordance with hydrogen bond interactions.

***Keywords*—**Laninamivir, neuraminidase, H5N1, pandemic H1N1, wild-type, MD simulation

I. Introduction

SINCE the 2009-pandemic H1N1 virus (pH1N1) and avian flu (H5N1) were widely spread out in various countries around the world and become resistant to the most common effective anti-influenza drug, oseltamivir [1, 2], leading to a worldwide public health problem. Recently, the new drug, laninamivir (R-125498), was approved and marketed in Japan in September, 2010 [3]. The long term inhibitory efficiency against neuraminidase (NA) of influenza A and B viruses as well as their mutations [3-5] is summarized in Table 1, however, its structure and dynamics properties inside the active site of the H5N1 and pH1N1 neuraminidases have not yet revealed. This is therefore the understanding of drug-target interaction and susceptibility of laninamivir in atomistic details becomes the goal of this present work.

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Neuraminidase is a glycoside hydrolase enzyme (EC 3.2.1.18) important for viral transmission to the host cell that is found on the surface of virus and plays a role to cleave the glycosidic bond of the terminal sialic acid from the host cell to permit new viral progeny releasing to infect the other cells. Besides, virus has another surface glycoprotein, hemagglutinin (HA), and M2 protein essential in influenza replication. HA initially attaches to the host cell receptor and enter to host organism. M2 protein is an ion channel protein that equilibrates the pH and involves in the fusion of viral and endosomal membranes. Two licensed adamantane drugs have been used to inhibit the M2 channel activity: amantadine and rimantadine. Unfortunately, the resistance of these adamantanes has been found with the high rate [6, 7].

TABLE I
THE 50% INHIBITORY CONCENTRATION (IC_{50}) VALUES OF LANINAMIVIR FOR VARIOUS INFLUENZA WILD-TYPE STRAINS OF NEURAMINIDASE

Virus strain	IC_{50} (nM)
A/H1N1 (seasonal flu)[5]	1.79
pH1N1[5]	1.83
A/H3N2[5]	2.13
Influenza B[5]	11-26
A/Yogohama/67/2006 (seasonal H1N1)[8]	3.03
A/Washington/29/2009 (pH1N1)[9]	1.57[a]
A/Hanoi/30408/05 (H5N1)[8]	0.32
A/Vietnam/1203/04 (H5N1)[8]	0.28

[a]FL (MUNANA) assay

To disclose the fundamental information of the laninamivir binding pattern in the H5N1 and pH1N1 NAs (Fig. 1), the molecular dynamics (MD) simulation was separately performed on the two drug-target systems. As a result, their global structures, hydrogen bonding interactions and decomposition of free energies per residue are relatively compared and discussed.

II. Materials and Methods

A. Initial Structures and Systems Preparation

The simulated systems of laninamivir inhibiting pH1N1 and H5N1 neuramiridases were prepared as follow. The Protein Data Bank (PDB), entry code 2HU4 was selected for H5N1 wild-type strain and then the oseltamivir structure was replaced by laninamivir. Meanwhile, the X-ray structure of pH1N1 taken from PDB code 3NSS with superimposed laninamivir was used. All system preparation and MD simulation steps were performed using Amber10 package

[10]. The ionizable amino acids such as Lys, Arg, Asp and Glu were categorized at *pH* 7.0. The protonation state of His was assigned agreement with hydrogen bond. For Cys, a possible disulfide bond was defined to stabilize the protein structures. The missing hydrogen atoms in systems were added using the LEaP module.

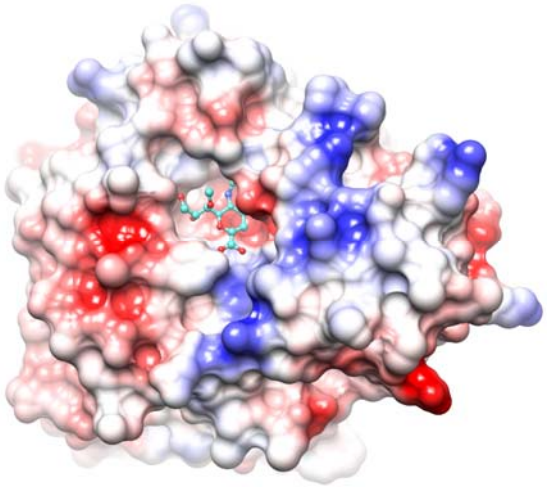

Fig. 1 The three-dimensional structure of laninamivir binding at the catalytic site of influenza A neuraminidase

Firstly, the minimization was performed on hydrogen atoms alone by 2000 steps of steepest descents (SD), and continued by 1000 steps of conjugated gradient (CG) to reduce bad steric interactions. Subsequently, each system was solvated by TIP3P waters with the minimum distance of 12 Å from the protein surface leading to the water box size of 89×87×95 $Å^3$ and 84×85×88 $Å^3$ for pH1N1 and H5N1, accordingly. The negative charges of all systems were neutralized by adding Na^+ ions. The modeled waters were then minimized by 2000 steps of SD and 1000 step of CG. In the last step, whole system was minimized with the same procedure.

B. Molecular Dynamics Simulations

The system was heated up to 300 K for 50 ps and subsequently simulated at 300 K for equilibration and production phases with a time step of 2 fs. The SANDER module in Amber and the periodic boundary with NPT ensemble at 1 atm was set. The SHAKE algorithm was used to constrain all bonds concerning hydrogen atoms. The cutoff distance for non-bonded interactions was set at 12 Å and the particle mesh Ewald method was applied to sufficient modify of long-range electrostatic interactions. The global root mean-square displacement (RMSD) results can see that the two systems tend to equilibrate at 3 ns. Therefore, the MD trajectories were extracted from the production phases of the last 7 ns.

III. Results and Discussion

To consider the stability of the two focused systems, the root mean-square displacement (RMSD) of the trajectories of H5N1 and pH1N1 NAs in complex with laninamivir from MD simulations relative to the initial structure were plotted and shown in Fig 2.

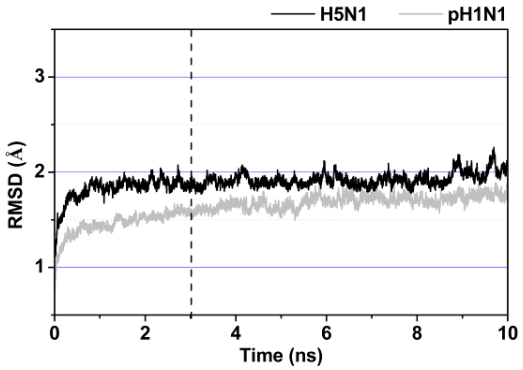

Fig. 2 The root mean-square displacements (RMSDs) of the H5N1 and pH1N1 NAs complexed with laninamivir

As a RMSD plot, the fluctuations for all atoms in the complexes are relatively similar and reach to equilibrium at 3 ns. Therefore, the MD trajectories from the last 7 ns were taken for analysis in terms of hydrogen bonding interactions and decomposition energy per residue basis.

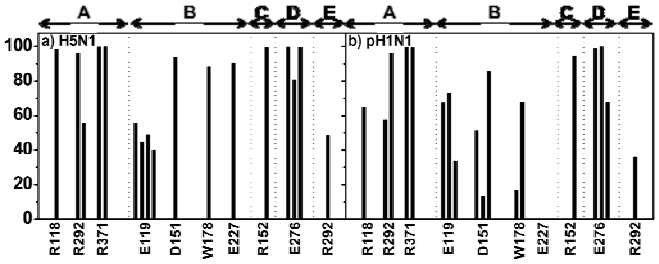

Fig. 3 Percent occupation pattern of hydrogen bonds formed from (A) carboxylate, (B) guanidinium, (C) N-acetyl, (D) trihydroxylpropyl, and (E) dihydropyran ring of laninamivir interacting with the NA residues of the two viruses: a) H5N1 and b) pH1N1

Hydrogen bonding interaction plays a critical role to stabilize protein-ligand complex. To further gain insight into the efficiency of the laninamivir binding to the H5N1 and pH1N1 NAs at the catalytic pocket, the percentage and number of hydrogen bond formation between this inhibitor and the surrounding NA residues were measured according to the subsequent criteria: *(i)* the distance between proton donor (D) and acceptor (A) atoms ≤ 3.5 Å; and *(ii)* the D-H…A angle ≥ 120°. The results are depicted in Fig. 3. The strong and moderate hydrogen bonding interactions are determined by hydrogen bond occupations higher than 75 and 50, respectively.

As seen in Fig. 3, the major hydrogen bonding interactions between inhibitor and the NA binding residues are quite similar among two complexes, although some amino acids were different at nearby the catalytic site. The oxygen atom of the carbonyl oxygen of the N-acetyl moiety (side chain C) and the dihydropyran ring (E) established strong and moderate

hydrogen bonding interactions with the guanidinium nitrogens of R152 and R292, respectively. The noticeable differences were detected at the carboxylate (A) and guanidinium (B) groups of ligand. The strong hydrogen bond interaction between trihydroxylpropyl group (D) of laninamivir and E276 was maintained.

At the guanidinium group (B), strong hydrogen bond with E227 was detected in H5N1 (Fig. 3a), while this of interaction was exhausted in pH1N1 (Fig. 3b). Again, the guanidinium nitrogen of laninamivir created a strong hydrogen bond with W178 of H5N1 complex meanwhile the moderate and rather weak interactions were found in the pH1N1. It is also worth to mention that this part of ligand formed moderate hydrogen bonds with residue E119 in both cases. This is in agreement with the experimental evidence showing that laninamivir is active against for both wild-type of H5N1 and pH1N1.

As expected, R371 contributes extensively towards hydrogen bonding interaction to the carboxylate moiety (A) of laninamivir in the two systems studied. However, the considerable differences were noticed at the R118. In H5N1 and pH1N1, the carboxylate oxygen atoms established strong and moderate hydrogen bonds with R292.

Taking into account the enzyme-inhibitor interaction, although hydrogen bonds between laninamivir and the NA binding residues were minor different in these two systems, the main interactions of ligand contributed by its contact amino acids were mostly conserved.

To identify the key residues for binding of laninamivir in the NA binding pocket of H5N1 and pH1N1 viruses, the interaction energy from each residue of NA contributed to laninamivir were calculated according to the MM/GBSA method [11] using the decomposition energy module of AMBER. Among all NA residues, the main contribution to laninamivir binding was from the catalytic residues (R118, D151, R152, R224, E276, R292, R371 and Y406) and the framework residues (E119, R156, W178, S179, D198, I222, E227, H274, E277, N294, and E425) as summarized in Fig. 4.

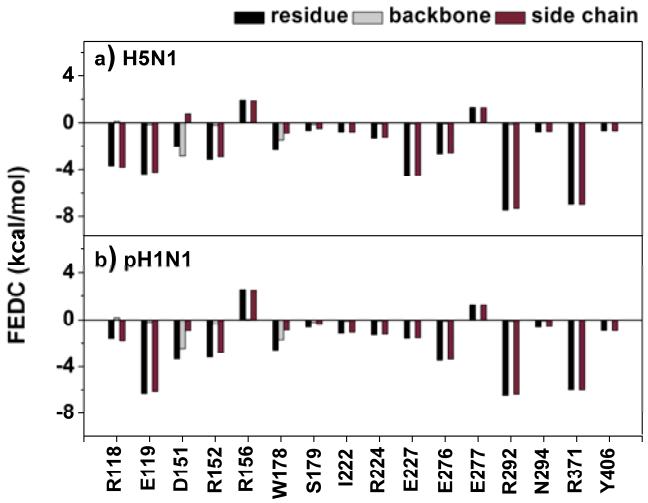

Fig. 4 The decomposition of free energy per residue at the binding site and framework site of the H5N1 and pH1N1 complexes

In Fig. 4, the interaction energy for both focused systems showed that almost all catalytic and framework residues (except for D151 and W178) provided the energy stabilizations through their side chains (brown histogram) in correspondence with the presence of drug-target hydrogen bonds (Fig. 3). Among these residues, the most contribution was likely gained from the inhibitor binding residues contacted through strong salt-bridge and hydrogen bond interactions with the drug charged groups. These residues are the R292 and R371 in catalytic site and the E119 (pH1N1) in framework site with binding energies of less than -6 kcal/mol. In addition, the residues R118, E119 (H5N1), D151, R152, W178 and E276 gave the energy contribution in the range of -2 to -6 kcal/mol.

IV. Conclusion

The MD simulations were applied to the susceptibility of laninamivir inhibiting to H5N1 and pH1N1 for wild type NA strain. All four side chains (carboxylate, guanidinium, N-acetyl, and trihydroxylpropyl) and trihydroxylpropyl ring of laninamivir provided the strong hydrogen bonding interactions with the catalytic and framework residues in the binding pocket of both viral strains. The positively charged guanidinium side chain of drug was well stabilized by the two negative charged residues, E119 and D151, as well as the W178 backbone similar to the previous study on the zanamivir [12-14]. Noticeably, the hydrogen formation via the oxygen atom of the dihydropyran ring with the guanidinium nitrogens R292 was only found in the laninamivir complexes. The important drug-target interactions by three arginines R118, R292 and R371, have extensively contributed to the conserved interaction in wild-type that found in all NA drugs [15-18]. Based on decomposition energy per residue basis, energy contributions are likely from the catalytic residues in the two studies systems. Taking into account hydrogen bonding formation and energy contribution, the laninamivir preferentially inhibits the viral influenza A subtypes H5N1 and pH1N1 through strong binding at the catalytic site of NA.

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