Biochemical and Biophysical Research Communications 470 (2016) 130–136

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

The 340-cavity in neuraminidase provides new opportunities for influenza drug development: A molecular dynamics simulation study

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ARTICLE INFO

Article history:
Received 18 December 2015
Accepted 3 January 2016
Available online 6 January 2016

Keywords:
Neuraminidase
Molecular dynamics simulation
340-cavity
Hotspot

ABSTRACT

Influenza neuraminidase (NA) is a pivotal target for viral infection control. However, the accumulating of mutations compromise the efficacy of NA inhibitors. Thus, it is critical to design new drugs targeted to different motifs of NA. Recently, a new motif called 340-cavity was discovered in NA subtypes close to the calcium binding site. The presence of calcium is known to influence NA activity and thermostability. Therefore, the 340-cavity is a putative ligand-binding site for affecting the normal function of NA. In this study, we performed molecular dynamics simulations of different NA subtypes to explore the mechanism of 340-loop formation. Ligand-binding site prediction and fragment library screening were also carried out to provide evidence for the 340-cavity as a druggable pocket. We found that residues G342 and P/R344 in the 340-loop determine the size of the 340-cavity, and the calcium ion plays an important role in maintaining the conformation of the 340-loop through contacts with G345 and Q347. In addition, the 340-cavity is predicted to be a ligand-binding site by metaPocket, and a sequence analysis method is proposed to predict the existence of the 340-cavity. Our study shows that the 340-cavity is not an occasional or atypical domain in NA subtypes, and it has potential to function as a new hotspot for influenza drug binding.

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1. Introduction

Influenza viruses have posted a great threat to human life since the last century [1]. Although yearly vaccines and antiviral drugs have been developed to treat influenza, the constantly mutating nature of viral strains weaken the power of antiviral strategies [2]. In order to efficiently treat this infectious disease, it is crucial to extensively explore drugs targeting hotspots in influenza viruses. Neuraminidase (NA), which facilitates viral shedding by cleaving terminal sialic acid residues on host cells, is one of the most promising drug targets [3]. Therefore, it is of great value to study in detail the structure and dynamics of NA for finding new binding sites and novel inhibitors to prevent and control influenza infections [4,5].

Based on phylogenetic distinction, NAs are divided into two groups: group-1 (N1, N4, N5, N8) and group-2 (N2, N3, N6, N7, N9) [6]. Recently, the crystal structure of N7, the last piece of NA "jigsaw" puzzle, has been solved [7]. The N7 structure highly resembles that of group-2 NAs. One characteristic in particular, an additional pocket adjacent to the active site of NA capped by the 340-loop (residues 342 to 347), was discovered in N7 as well as in N6. This pocket is called the 340-cavity [7]. The 340-loop of N7/N6 is oriented further away from the conserved calcium ion binding site, in contrast to other NA subtypes. Sequence analysis showed N7/N6 has a serine instead of proline in position 326, and its interaction with the 340-loop is suspected to be a key factor in maintaining the unique 340-loop conformation in N7/N6. However, it is not clear whether other factors contribute to the special 340-loop conformation. The dynamical properties of the 340-loop in N7 and other group-2 NAs (i.e., N2) have never been experimentally observed. Furthermore, use of the 340-cavity for rational structure-based drug design has not yet been explored.

Inspired by the above challenges, normal molecular dynamics (MD) simulations were performed on the N2 and N7 subtypes to

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http://dx.doi.org/10.1016/j.bbrc.2016.01.007
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explore the mechanism for 340-cavity formation. Additionally, ligand-binding site prediction and fragment library screening were also carried out to test the suitability of 340-cavity for ligand targeting. Our study finds that the unique sequence combination of the N7 340-loop, the calcium ion binding pattern, and the salt-bridge and hydrogen bond interactions between 340-loop and nearby residues contribute to the special conformation of the N7 340-loop. Moreover, the 340-cavity was also predicted to be a ligand-binding site, and a ligand binding pattern was also produced. We posit that this new structural understanding of the 340-loop can be applied to design novel inhibitors of NA enzymes.

2. Methods

2.1. MD simulation details

Normal MD simulations of N2 (1NN2) and N7 (4QN3) were performed. The homotetrameric conformation of NA allows us to take advantage of multi-copy sampling, and the simulation time for all the NA protomers in each system was 200 ns? Both of N2 and N7 systems were solvated with TIP3P waters in an octahedral box [8]. Sodium and chloride ions were added with a concentration of 100 mM, and the conserved calcium ions were preserved in the right positions in the NA structure. The GROMACS program suite version 4.5.7 and Amber99SB force field were used in all simulations [9]. The simulations were performed in an isothermal-isobaric ensemble (300 K, 1 bar). Bond length constraints were applied to all bonds that contained hydrogen atoms based on the LINCS protocol [10]. An integration step of 0.002 ps was used in the simulations. Electrostatic interactions were treated with Particle Mesh Ewald method with a cutoff of 0.9 nm with grid spacing for the FFT grid <0.12 nm [11].

2.2. Fragment library screening

We used the cluster center from dihedral principle component analysis (dPCA) as the receptor [12], and the fragment library was comprised of 7645 compounds provided by ChemBridge (Divers E set, Chembridge Chemical). For the receptor, we deleted solvent and added hydrogen via Chimera software [13], created the corresponding molecular surface via DMS, represented potential binding sites via the SPHGEN utility, and selected the subset of spheres that centered on the 340-cavity via SPHERE_SELECTOR. The GRID utility was used to calculate the grid of the receptor, and fragment library screening was performed using DOCK6 [14].

2.3. Analysis methods

340-cavity volume calculation

The volume of the 340-cavity was calculated by POVME [15]. The NA structure snapshots were extracted from the trajectory every 2 ps and superimposed onto the reference structure with a pre-generated 3D-grid representing the 340-cavity. The volume was calculated by counting the grid points located in the 340-cavity.

Dihedral principal components analysis (dPCA)

Before dPCA [12], all backbone dihedral angles of the 340-loop were collected from trajectories every 1 ps. dPCA was performed on these components. All calculated snapshots were projected to the first two eigenvectors to get the first two principal components to make a two-dimensional free energy landscape.

Ligand-binding site prediction

Ligand-binding site prediction was performed by metaPocket, which combines LIGSITE, PASS, Q-SiteFinder, and SURFNET to improve prediction success rate by using its default parameter [16]. The structure of the dPCA cluster center was used, returning 7 potential ligand-binding sites.

3. Results

3.1. Volume of the 340-cavity

In order to explore the conformational flexibility of the 340-loop during the simulation, the volume of the 340-cavity, one of the most important reaction coordinates that reflects the dynamics of the 340-loop, was calculated. The potential of mean force (PMF) as a function of 340-cavity volume was constructed (Fig. 1A). Two local minima in the PMF as a function of 340-cavity volume around 50 $Å^3$ and 180 $Å^3$ were identified in N7 (red line), henceforth denoted minima 1 and minima 2, respectively. The first local minima with volume 51 $Å^3$ is associated with the lowest free energy, indicating that this configuration is the most favorable in N7. This conformation is nearly the same as the crystal structure based on volume size comparison. Moreover, the second free energy minimum with volume 177 $Å^3$ suggests that the 340-cavity opens to a larger extent in N7. In contrast, the free energy minimum at 0 $Å^3$ in N2 indicates that almost no 340-cavity forms during simulation (black line). The PMF maps illustrate that the 340-loop prefers open 340-cavity conformations in N7 system and closed 340-cavity conformations in N2.

3.2. Turn A gauges 340-cavity volume in N7

To further characterize the conformation changes of the 340-loop, dihedral principal components analysis (dPCA) was performed. The free energy landscapes of the N2 and N7 systems generated by dPCA are distinct (Fig. 1B,C), suggesting that the 340-loop in two systems prefer disparate configurations. Identical to the PMF volume calculation, two local minima were discovered from dPCA in N7 system, suggesting that the 340-loop in N7 has two favored conformations. Clustering analysis provided a representative structure for each local minimum in N7. Fig. 1E,F shows the cluster centers for minima 1 and 2, illustrating the 340-cavity with different volume sizes.

Backbone dihedral angle analysis was applied to decipher structural alterations of the N7 340-loop. After computing the backbone dihedral angles from structure snapshots of the two local minima of the dPCA free energy landscape of the N7 340-loop residues (Fig. 1C), the backbone dihedrals of G342 and P344 were identified to have the largest difference between the two local minima. G342 had an average phi value of 139.56° and −80.73° and an average psi value of 139.19° and −73.47° in local minima 1 and 2, respectively. The average psi values for P344 were 31.17° and 149.61°, respectively.

According to the shape of the 340-loop in NA subtypes, we divided the 340-loop into two parts, and named them turn A (residues 342–344) and turn B (residues 345–347). After superimposing these two cluster centers together, turn A of the 340-loop was shown to have much greater variation than turn B, and G342 in turn A formed one hydrogen bond with Q296 in local minimum 2 (Fig. 2B). However, this interaction was absent in the first local minimum. The probability of forming a hydrogen bond between G342 and Q296 was only 7% in the initial 10 ns of the simulation, but this value increased to 23% in the last 20 ns (Table S1). In conclusion, open conformations of N7 are gauged by turn A of the 340-loop and stabilized by the hydrogen bond formed between G342 and Q296.

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