Molecular dynamics investigation of the pH-dependent influenza hemagglutinin conformational changes

Shadi A Badiee, Vivek Govind Kumar, Adithya Polasa and Mahmoud Moradi

Department of Chemistry and Biochemistry, University of Arkansas, Fayetteville, AR, USA.

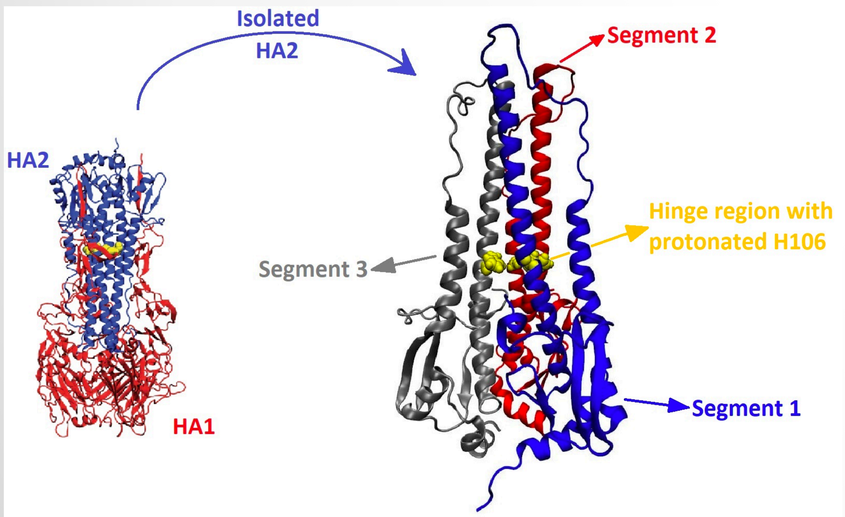
Introduction

Hemagglutinin (HA) is a homotrimeric glycoprotein located on the surface of influenza virus that mediates fusion between viral and endosomal membranes of the host cell. While influenza viruses have been very widely studied and are well characterized as a global health concern, the atomistic details of HA-mediated membrane fusion are poorly understood.

The pre-fusion form HA0 is trimeric and consists of three identical monomers. HA0 is proteolytically cleaved into HA1 and HA2 glyco-polypeptides. HA2 functions as the membrane fusion domain while HA1 recognizes glycolipids and glycoproteins with sialic acid moieties on the host cell surface to trigger endocytosis. Acidification of the endosome causes large-scale irreversible conformational changes in HA, while HA1 moves away from the HA2 domains, allowing the refolding of the HA2 loop at a hinge region and releasing the fusion peptide.

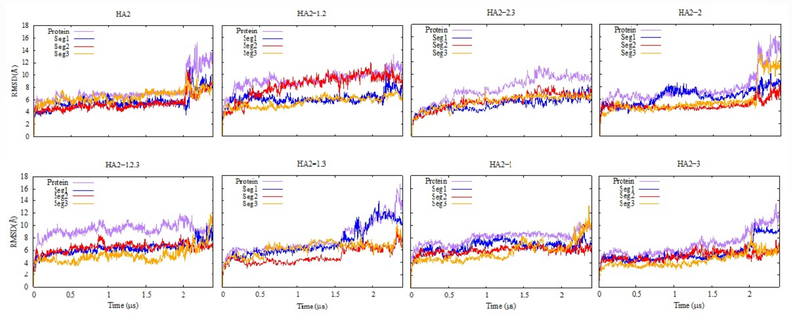
In this study, we have investigated the conformational changes of HA2 trimer in the absence of HA1 under various pH conditions through protonation of a specific Histidine residue (H106) located in the hinge region of HA2 using all-atom microsecond-level equilibrium MD simulations.

Structure of HA and isolated HA2

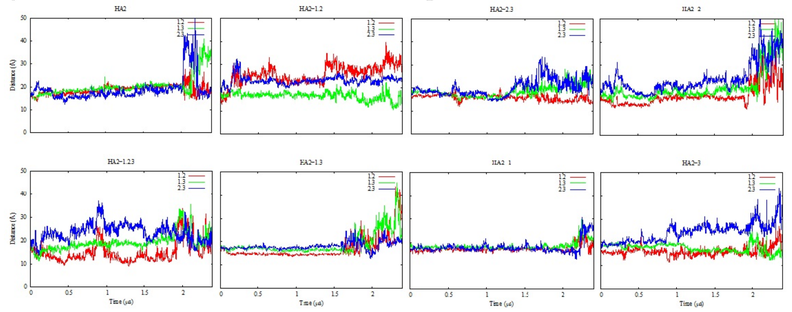


1. X-ray crystal structure H3 influenza hemagglutinin (5KUY) and isolated HA2. HA is proteolytically cleaved into HA1 and HA2 glycopolypeptides. HA1 functions as a receptor binding subunit while HA2 is responsible for membrane fusion. HA2 has three identical segments and H106 is protonated on hinge region of these segments.

Results



2. RMSD. Preliminary simulations with isolated HA2 trimers. The protonation of conserved Histidine on HA2 is done in 0, 1, 2 and 3 H106 residues on different segments of HA2 and builds 8 different systems. Our preliminary data does not indicate a major conformational change within the timescale of the simulation (2.4 μs); however, longer simulations may indicate a differential dynamic behavior between different protonation states.



3. Interhelical Distance. Although the protonation of conserved histidine at the HA2 hinge region of all systems could result in conformational changes, the interhelical distance in the system with three protonation is occurring faster compared to the other systems such as 0, 1 and 2 protonation.

		Occupancy %							
Donor	Acceptor	HA2	HA2-1.2.3	HA2-1	HA2- 2	HA2-3	HA2-1.2	HA2-2.3	HA2- 1.3
ARG453-Side-2	ASP461-Side-3	56.04	44.80	0.59	2.57	43.76	33.05	21.34	40.78
LYS446-Side-2	GLY330-Main-3	52.96	21.76	3.42	6.52	9.78	5.76	2.19	10.57
ASP461-Side-3	ARG453-Side-1	136.42	51.47	39.45	41.11	21.39	40.04	5.48	37.99
GLU410-Side-2	ARG405-Side-1	97.34	93.43	88.09	37.85	90.61	95.37	88.63	89.23
GLU414-Side-3	TYR412-Side-2	12.43	45.78	13.96	44.17	47.92	47.97	44.17	41.87
HSP435-Side-1	ASP438-Side-2	-	58.62	22.95	0	0	65.92	0	12.51
ASP438-Side-1	HSP435-Side-3	-	11.07	0	0	0	0	70.69	48.33
HSP435-Side-2	ASP438-Side-3	-	42.77	0	3.19	9.4	18.85	5.69	0

4. Table of inter protomer Hydrogen-bond interactions. Almost all of the hydrogen-bond interactions are present in all of the systems such as protonated and non-protonated systems and they are unchanged. Moreover, in all protonated systems, the best interaction is between protonated His (HSP) and residue ASP438.

Methods

Equilibrium MD simulations were based on the X-ray crystal structure of H3 influenza hemagglutinin (5KUY – 2.598A) (Figure 1).CHARMM-GUI was used to generate eight different models including: 1) H106 unprotonated on all 3 HA2 chains; 2) H106 protonated on all 3 HA2 chains 3)H106 protonated on 1 HA2 chain(3 models); 3) H106 protonated on all 2 HA2 chains(3 models). The models were solvated in a rectangular water box and 150 mM of sodium and chloride ions were inserted. System size was approximately 237800 atoms for each model. Production runs were carried out in an NPT ensemble at 310 K using an integrator time step of 2 fs. The initial production runs for each model lasted 15 ns and these were extended to 2.4 microseconds on Stampede2 and Anton2 (Pittsburgh Supercomputing Center, D.E.Shaw Research).

Conclusion

Based on X-ray crystal structures and biochemical evidence, several mechanisms have been proposed for HA-mediated membrane fusion . The most widely accepted model suggests that HA2 undergoes extensive conformational refolding. Acidification of the endosome causes the HA1 domains to move away from the HA2 domains, allowing the loop-to-helix transition.

Our microsecond-level equilibrium trajectories reveal that even the isolated HA2 with an only one single protonation undergoes conformational changes and dissociation of HA1 is enough to trigger conformational changes. The most varying conformational changes is the system with three protonation.

However, longer simulations is needed to indicate a differential dynamic behavior between different protonation states.

References

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2) Kalani MR. Moradi A, Moradi M, Tajkhorshid E. 2013. Characterizing a histidine switch controlling pH-dependent conformational changes of the influenza virus hemagglutinin. Biophys. J. 105:993-1003.

Acknowledgments

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