Characterization of evolutionary changes in hemagglutinin of influenza H1N1 virus: a computational analysis

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Abstract Influenza virus continues to evolve due to changes in the genome and the new strain of virus is more pathogenic than the previous strain. These changes may also help the virus to cross species barrier and may also affect the binding pattern of virus. The main theme of the current study is the identification of changes in the hemagglutinin sequence of H1N1 virus from 1960 to 2011 and also how these changes affect the binding properties of virus. From 1960 to 2000 following important changes were observed: Ala198Asp and Gly225Glu in 1980; and Gly225Asp in 1999. From 1999 to 2011 many changes were observed, most of the changes were transient, but two of the changes, Gly225Asp and Ala227Glu, were consistent in the period of 1999–2010. These residues make the binding stronger. The important conserved residues are Asp190, Tyr98, His183 and Gln226. The current study will provide an understanding how virus evolve with the passage of time. The current study also helps to understand the changes in the binding pattern of virus. It will also help for the identification of new therapeutic targets.

Keywords Hemagglutinin · Neuraminidase · Sialic acid

Introduction

Influenza is a highly contagious, airborne respiratory tract infection in mammals and birds [6]. It results in significant disease burden during seasonal influenza outbreaks every year [2]. According to WHO report the influenza is responsible for 250,000–500,000 deaths every year around the world (WHO report, 2010). The 1918 influenza A virus (H1N1) pandemic caused a death toll of 40 to approximately 50 million [3] which was one of the most devastating disease outbreaks in human history.

Influenza viruses are members of family orthomyxoviridae are enveloped, single stranded RNA viruses [13]. The influenza genome is segmented, with eight genetically independent RNA segments and encodes ten proteins. These proteins include two functional surface glycoproteins hemagglutinin (HA, attaches the virus to the host cell for cell entry) and neuraminidase (NA, facilitates the spread of the progeny virus), internal structural proteins, nucleocapsid protein (NP), and two matrix proteins (M1 and M2) [7, 13]. The polymerase of this RNA virus lacks proof reading activity, which gives rise to considerable viral variability culminating in three different types A, B and C, in addition to many subtypes based on variations in HA and NA [21].

There are sixteen antigenically different HA (H1–H16) and nine different NA (N1–N9) types have been reported so far, and different combinations of HA and NA types determine the subtype of the virus (e.g. H1N1, H5N1, H9N2, or H7N7) [25]. Genetic reassortment of influenza A virus within different hosts (including avian and swine), and antigenic shifts and drifts in the HA and NA proteins,

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are believed to be important factors causing serious outbreaks and pandemics of 1918, 2005, and 2009 [10, 16].

With reference to human transmission, three subtypes of influenza virus, namely H1N1, H2N2 and H3N2, are known [18, 24, 28]. Among them, H1N1 is maintaining a profile to persistently infect humans at random intervals since 1918. The pandemic of 1918 of H1N1 was a significant source of mortality globally [24]. The H1N1 subtype of influenza A virus was responsible for the 1918–1919 Spain pandemic infecting 5 % of the world population and resulting in 50 million deaths worldwide [22]. It reappeared in 1977 and is known to be circulating sporadically in the population till now. In this context, H1N1 possess a unique historical background which puts it as a good model to understand cross specie transmission [24, 28].

The recent pandemic of the (July 2009 H1N1) influenza A virus (also called swine or Mexican flu) was highly pathogenic as a new swine flu strain, naturally hosted by pigs, crossed the species barrier to infect humans and, acquired the capability for human to human transmission. The continuous evolution of the influenza virus to become a more efficient human pathogen may enable it to produce a major pandemic hence making it a constant threat to human health.

The HA of influenza virus interacts with host cell receptors present in the respiratory tract of human and pigs containing the terminal sialic acid (SA) residue [26], leading to cellular entry through endocytosis. SAs are usually found either as α -2, 3 or as α -2, 6 linkages to galactose (Gal) [20]. In case of swine respiratory tract, the SA- α -2, 6-Gal receptor is dominant in the epithelium of the upper tract (trachea and bronchus), while there is a relative increase of SA- α -2, 3-Gal receptor towards the lower tract (bronchiole and alveolar region). In the human trachea, on ciliated and non-ciliated epithelial cells SA- α -2, 6-Gal receptor is abundantly expressed and SA- α -2, 3-Gal receptor is only sparsely present on non-ciliated cells [12]. Swine H1N1 can cross specie barrier and cause infection to humans due to the presence of SA- α -2, 3-Gal and SA- α -2, 6-Gal receptor in the respiratory tract of human and swine. In human respiratory tract HA preferentially binds to SA- α -2, 6-Gal whereas in avian and swine respiratory tract HA preferentially binds to SA- α -2, 3-Gal receptor [8].

Specificity of HA of influenza virus determines host range restriction [23]. Emergence of new mutations in this region may change the host range restriction and may consequently leads to sporadic human infections of Influenza virus [4]. Moreover, these mutations may change its binding affinity with human receptor (SA- α -2, 6-Gal). Therefore, it is very imperative to understand the variation in HA structure concerning receptor binding with reference to evolutionary timeline. This study is therefore designed to computationally characterize the changes in the HA protein

of H1N1 virus, between the years 1918 and 2011, and their effect on its binding with the human SA- α -2, 6-Gal receptor. The current study will aid in understanding some determinants of viral attachment, and may also serve as a novel vaccine candidate to target highly conserved regions.

Materials and methods

Collection of sequences

The protein sequences of HA (125 sequences) from influenza viruses of the swine H1N1 from 1960 to 2011 were downloaded using the advanced database search at the NCBI's Influenza Virus Resource and uniprotKB.

Multiple alignment and conservation analysis

Sequence analysis was performed to ascertain the changes in the receptor binding domain of H1N1-HA. For multiple sequence analysis (MSA) the sequences were divided in five groups having sequences of a span of 10 years for each group and each group contains 25 sequences of HA. This categorization facilitate the management of the data, allowed the identification of diversity in the sequences based on the year they were isolated, along with the comparative mutational analysis followed by the calculation of conservation percentage. HA sequence of swine H1N1 detected in 1930 (UniProtKB: Q9WCD9) was used as a reference for all other sequences of HA. Sequence analysis was carried out following an established protocol using ClustalX [14]. The resulting multiple alignment was visualized and annotated in Jalview [27].

Ligand building

SA- α -2, 6-Gal was built and energy minimized under MM2 force field [1] by ChemBioOffice 2010.

Docking studies

The optimized SA- α -2, 6-Gal structure was docked into the binding site of 1930-H1N1-HA (PDB ID: 1RUY) in order to reproduce the crystal structure results. Automated docking studies were performed using the genetic algorithm GOLD (Genetic Optimization for Ligand Docking) (v. 3.1; CCDC, Cambridge, UK). The algorithm had been previously validated and successfully tested on a data set of over 300 complexes extracted from the PDB. The binding site was initially defined as all residues of the target within 10 Å resolution centered on the proteins active site. Chemscore was chosen as energy scoring function and the standard default settings were used in all calculations.

Rescoring was performed for the evaluation of fitness function using GOLD score.

For each of the 50 independent genetic algorithm runs, a default maximum of 10,000 genetic operations was performed, using the default operator weights and a population size of 100 chromosomes. Default cutoff values of 2.5 Å for hydrogen bonds and 4 Å for Van der Waals interactions were employed. Carboxylate and carboxamide substituents on aromatic rings were allowed to rotate. Early termination was allowed for results differing by <1.5 Å in ligand all atoms RMSD. The parameters that were used in docking of 1930-H1N1-HA with SA- α -2, 6-Gal were taken as standard for further docking studies.

Sequence analysis suggested important changes in the 130 loop, 190 helix and in 220 loop, known to be involved in receptor binding. These changes were incorporated into the crystal structure (PDB ID: 1RUY) using Molecular Operating Environment (MOE). Docking studies were repeated using same parameters as used for docking of 1930-H1N1-HA with SA- α -2, 6-Gal. Post docking analysis was done using PyMOL and MOE in order to evaluate close contacts such as ionic, hydrogen bond, hydrophobic and Van der Waals interactions.

Electrostatic potential of HA

The electrostatic potential of active site of HA was calculated through Delphi and PyMOL.

Results

Multiple sequence analysis

The receptor binding domain was identified as described by Gamblin et al. [9]. The active site consist of three secondary structure elements—the 190 helix (residues 190–198), 130 loop (residues 135–138), and 220 loop (residues 221–228) (Fig. 1).

The multiple sequence analysis (MSA) of the HA active site residues showed few changes in 130 loop, 190 helix and in 220 loop (Supplementary Table I) between the years 1960 and 2000 which include: Ala198Asp and Gly225Glu in 1980; and Gly225Asp in 1999. From 2001 onwards to 2011 (Fig. 2), numerous changes were observed in 130-loop, 190-helix and in 220-loop replacing non-polar residues by polar residues. All the changes were of transient nature, the changed residues revert back to its native residue, but two changes, Gly225Asp and Ala227Glu, were consistent in the period of 1999–2010.

Fig. 1 The receptor binding domain of HA-H1N1 (Pdb ID: 1RUY). Figure generated through PyMOL

Docking of SA- α -2, 6-Gal into H1N1-HA crystal structure

All the years (1980, 1983, 1989, 1999, 2001, 2002, 2003, 2005) in which changes were observed in receptor binding domain and also the years in which H1N1 pandemics (1918, 1930, 2009) had occurred were docked with SA- α -2, 6-Gal (Supplementary Table II).

Figure (3a) shows the docking pose (3rd in ranking by chemscore), generated by GOLD, of SA- α -2, 6-Gal in the active site of the 1930-H1N1-HA crystal structure (PDB ID: 1RUY), reproducing the binding conformation present in the original crystal structure [9]. This complex shows nine strong hydrogen bonds (<3 Å) between SA- α -2, 6-Gal and seven HA active site residues, Val135, Thr136, Ala137, Tyr95, Lys222, Gly225 and Gln226. As this docking pose was consistent with the experimental structure showing binding of SA- α -2, 6-Gal and H1N1-HA, same docking parameters in GOLD were used for further docking studies.

Figure (3b) showing the docking pose, generated by GOLD, of SA- α -2, 6-Gal in the active site of the 2009-H1N1-HA (UniProtKB: C6KQW0) structure. There were 13 strong hydrogen bonds (<3 Å) with nine residues, Val135, Thr136, Ala138, Tyr95, Lys222, Asp225, Gln226 and Glu227 of H1N1 HA, as judged by the GOLD program. Three positions in the receptor binding domain which are conserved and not transformed from 1960 to 2011 are Thr136, Lys222 and Gln226 and these residues are also involved in binding with SA- α -2, 6-Gal.

2WRG-1918	130-Loop	190-Helix
1RUY-1930	TTKGVTAACS	TTKGVTAACS
mC5MUU912001	YAGASSFY	YAGASSFY
mA8D6V612002	RNL LWL	RNL LWL
mQ1PTK312003	VKKGNSYP	VKKGNSYP
mC4RTF312004	KL SKSY	KL SKSY
mC4WWU212005	INNKKEVL	INNKKEVL
mA6Z1Z012006	IWG	IWG
mC5MUU6012007	HPPTSD	HPPTSD
mC7AGK912008	DDQTL	DDQTL
mC6CWM012009	YQNNHT	YQNNHT
mD5X15112010	YVSV	YVSV
mF0T1U9412011	VT	VT
2WRG-1918	VSASCSHNGK	VSASCSHNGK
1RUY-1930	SSFYRNL	SSFYRNL
mC5MUU912001	LWL	LWL
mA8D6V612002	TEKNGSYP	TEKNGSYP
mQ1PTK312003	NL SKSY	NL SKSY
mC4RTF312004	YVNNKERE	YVNNKERE
mC4WWU212005	VLWGVHHP	VLWGVHHP
mA6Z1Z012006	SNIGQRA	SNIGQRA
mC5MUU6012007	YHTENAYVS	YHTENAYVS
mC7AGK912008	VT	VT
mC6CWM012009	TRGTTVAC	TRGTTVAC
mD5X15112010	SHSGANSF	SHSGANSF
mF0T1U9412011	YRNL LWI	YRNL LWI
2WRG-1918	VKKGNSYP	VKKGNSYP
1RUY-1930	KL SKSY	KL SKSY
mC5MUU912001	YVNNKERE	YVNNKERE
mA8D6V612002	VLWGVHHP	VLWGVHHP
mQ1PTK312003	PTDSVQ	PTDSVQ
mC4RTF312004	QTL	QTL
mC4WWU212005	YQNNHT	YQNNHT
mA6Z1Z012006	YVSV	YVSV
mC5MUU6012007	VT	VT
mC7AGK912008	TRGTTVAC	TRGTTVAC
mC6CWM012009	SHSGANSF	SHSGANSF
mD5X15112010	YRNL LWI	YRNL LWI
mF0T1U9412011	VKKGNSYP	VKKGNSYP
2WRG-1918	KL SKSY	KL SKSY
1RUY-1930	YVNNKERE	YVNNKERE
mC5MUU912001	VLWGVHHP	VLWGVHHP
mA8D6V612002	PTDSVQ	PTDSVQ
mQ1PTK312003	QTL	QTL
mC4RTF312004	YQNNHT	YQNNHT
mC4WWU212005	YVSV	YVSV
mA6Z1Z012006	VT	VT
mC5MUU6012007	TRGTTVAC	TRGTTVAC
mC7AGK912008	SHSGANSF	SHSGANSF
mC6CWM012009	YRNL LWI	YRNL LWI
mD5X15112010	VKKGNSYP	VKKGNSYP
mF0T1U9412011	KL SKSY	KL SKSY
2WRG-1918	YVNNKERE	YVNNKERE
1RUY-1930	VLWGVHHP	VLWGVHHP
mC5MUU912001	PTDSVQ	PTDSVQ
mA8D6V612002	QTL	QTL
mQ1PTK312003	YQNNHT	YQNNHT
mC4RTF312004	YVSV	YVSV
mC4WWU212005	VT	VT
mA6Z1Z012006	TRGTTVAC	TRGTTVAC
mC5MUU6012007	SHSGANSF	SHSGANSF
mC7AGK912008	YRNL LWI	YRNL LWI
mC6CWM012009	VKKGNSYP	VKKGNSYP
mD5X15112010	KL SKSY	KL SKSY
mF0T1U9412011	YVNNKERE	YVNNKERE
2WRG-1918	VLWGVHHP	VLWGVHHP
1RUY-1930	PTDSVQ	PTDSVQ
mC5MUU912001	QTL	QTL
mA8D6V612002	YQNNHT	YQNNHT
mQ1PTK312003	YVSV	YVSV
mC4RTF312004	VT	VT
mC4WWU212005	TRGTTVAC	TRGTTVAC
mA6Z1Z012006	SHSGANSF	SHSGANSF
mC5MUU6012007	YRNL LWI	YRNL LWI
mC7AGK912008	VKKGNSYP	VKKGNSYP
mC6CWM012009	KL SKSY	KL SKSY
mD5X15112010	YVNNKERE	YVNNKERE
mF0T1U9412011	VLWGVHHP	VLWGVHHP
2WRG-1918	PTDSVQ	PTDSVQ
1RUY-1930	QTL	QTL
mC5MUU912001	YQNNHT	YQNNHT
mA8D6V612002	YVSV	YVSV
mQ1PTK312003	VT	VT
mC4RTF312004	TRGTTVAC	TRGTTVAC
mC4WWU212005	SHSGANSF	SHSGANSF
mA6Z1Z012006	YRNL LWI	YRNL LWI
mC5MUU6012007	VKKGNSYP	VKKGNSYP
mC7AGK912008	KL SKSY	KL SKSY
mC6CWM012009	YVNNKERE	YVNNKERE
mD5X15112010	VLWGVHHP	VLWGVHHP
mF0T1U9412011	PTDSVQ	PTDSVQ
2WRG-1918	QTL	QTL
1RUY-1930	YQNNHT	YQNNHT
mC5MUU912001	YVSV	YVSV
mA8D6V612002	VT	VT
mQ1PTK312003	TRGTTVAC	TRGTTVAC
mC4RTF312004	SHSGANSF	SHSGANSF
mC4WWU212005	YRNL LWI	YRNL LWI
mA6Z1Z012006	VKKGNSYP	VKKGNSYP
mC5MUU6012007	KL SKSY	KL SKSY
mC7AGK912008	YVNNKERE	YVNNKERE
mC6CWM012009	VLWGVHHP	VLWGVHHP
mD5X15112010	PTDSVQ	PTDSVQ
mF0T1U9412011	QTL	QTL
2WRG-1918	YQNNHT	YQNNHT
1RUY-19		

potential of 1930-HA (PDB ID: 1RUY) and 2009-HA (UniProtKB: C6KQW0). In 1930-HA the surface is showing highly electropositive groove which is gradually moving towards the electronegativity due to changes in the virus. And in 2009-HA it becomes highly electronegative.

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Fig. 3 The docking conformation of hemagglutinin. SA- α -2, 6-Gal into the receptor site of **a** 1930-H1N1 HA and **b** 2009-H1N1 HA. The figure is generated through PyMOL V1.3

Fig. 4 Electrostatic potential of **a** 1930-HA and **b** 2009-HA calculated through Delphi. The negative charge in the pocket represented by red color while positive charge represented by blue color. The ligand (SA- α -2, 6-Gal) shown in stick form

Discussion

Influenza H1N1 virus is a subtype of influenza A virus, was the most common cause of human influenza (flu) in 2009. The virus has undergone different changes with passage of time rendering it to be more virulent. Current study was targeted to identify the changes in HA protein of H1N1. The evolution of HA of H1N1 was studied over the period of 50 years from 1960 to 2011. From 1960 to 2000 following important changes were observed: Ala198Asp and Gly225Glu in 1980; and Gly225Asp in 1999. As Ala and Gly both are neutral residues while Glu and Asp are charged residues. So if these residues involved in binding they make the binding strong with the human receptor. From 1999 to 2011 many changes were observed in 130-loop (position 135, 137 and 138), 190-helix (position 193, 196, 198) and 220-loop

(position 222) but these changes were transient i.e. revert back to the native amino acid. The two important changes observed over this period were Gly225Asp and Ala227Glu, these are permanent changes i.e. once change never revert back to native amino acid. These two changes are important as Gly and Ala both are neutral residues replaced by acidic and charged residues.

The effect of these changes was also studied through docking analysis. For docking the 1930-H1N1 (PDB ID: 1RUJ) was taken as reference. The binding of H1N1 with SA- α -2, 6-Gal was reproduced and the parameters used for this docking run was used as standard for further docking runs. The interactions of 1980 (UniProtKB: Q9WCE5), 1983 (UniProtKB: C1FWA8), 1989 (UniProtKB: C1FWE1), 1999 (UniProtKB: A8D6U8), 2001 (UniProtKB: C5MUA9), 2002 (UniProtKB: A8D6V6), 2003 (UniProtKB: Q1PTK3), 2005

(UniProtKB: C4NWI2) and 2009-HA (UniProtKB:C6KQW0) with SA- α -2, 6-Gal was studied in detail. The results showed that in the years where Gly225Asp change was observed the binding gets strong. In 2009-HA the two important residues that are involved in binding are Asp225 and Glu227 were present. The involvement of these two residues makes the binding stronger as determined through number of bonds as well as through docking scores. At 225 and 227 position neutral residues (Gly and Ala) were present initially as the virus evolves these residues replaced by highly negatively charged acidic residues that make more H-bond with SA and GAL moiety of receptor. Besides other environmental factors this may be one of the factors of 2009 pandemic. Reid et al. [19] in 2003 also identified that the Asp190 and Asp225 were the main cause of 1918-H1N1 pandemic. It has also been reported in literature that the involvement of these two residues in the binding were the cause of 2009 pandemic [17]. While studying the cause of 2009-pandemic, a group of scientists find out that Glu227 was one of the cause of this pandemic [5].

The electrostatic surface of 1930-HA shows an electropositive groove and this electropositivity continues till the year 1999, after year 1999 the electropositivity starts reducing due to changes in the amino acid sequence and in 2009 the active site become totally electronegative. This electronegativity is implicated to be due to the presence of highly negatively charged residues Asp and Glu in the active site. This electronegative surface able to make strong H-binding with electropositive atoms of ligand. The changes in the electrostatic potential of active site of HA provides another evidence of how virus evolve over the period of time due to addition of negatively charged residues (Asp225 and Glu227) in the active site.

From sequence analysis and docking studies the important conserved residues identified are Asp190, Tyr98, His183 and Gln226. It was found in previous studies that Tyr98 was found to be conserved in most influenza A HAs (H3, H9, H5) [11]. It has been previously reported that Glu190 to Asp mutations in the H1 HA appeared important for the increased affinity of the viruses for SA-a-2, 6-Gal [15]. In the current study the Asp190 is conserved which is again providing an evidence of strong affinity of H1N1 with the human receptor. The conserved residues are important from the therapeutic point of view i.e. if new treatment strategy will be devised for swine H1N1 then these residues might be a good target.

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