Monatsh Chem (2010) 141:801–807
DOI 10.1007/s00706-010-0319-z

ORIGINAL PAPER

Molecular insights into human receptor binding to 2009 H1N1 influenza A hemagglutinin

Nadtanet Nunthaboot · Thanyada Rungrotmongkol · Maturos Malaisree · Panita Decha · Nopporn Kaiyawet · Pathumwadee Intharathep · Pornthep Sompornpisut · Yong Poovorawan · Supot Hannongbua

Received: 25 September 2009 / Accepted: 2 May 2010 / Published online: 27 May 2010
© Springer-Verlag 2010

Abstract The current pandemic of the viral 2009 H1N1 influenza and its sustained human–human transmission has raised global concern for human health. The binding of the viral glycoprotein hemagglutinin (HA) and the human α-2,6-linked sialopentasaccharide (SIA-2,6-GAL) host cell receptor is a critical step in the viral replication cycle. Here, the complex structure of the 2009 H1N1 HA bound to the SIA-2,6-GAL sialopentasaccharide receptor was constructed by using homology modeling and molecular dynamic simulations. The receptor was found to fit very well within the HA binding pocket and formed hydrogen bonds with the residues of the 130-loop, 190-helix, and 220-loop. Most receptor binding residues play a significant role in stabilizing the protein–receptor complex with major contributions being provided by V135, T136, A137, K222, and Q226. The results are similar to the human SIA-2,6-GAL sialopentasaccharide receptor binding to H1 HA subtype, but are slightly different from those of H3, H5, and H9 HAs.

Keywords Computational chemistry · Hydrogen bonds · Molecular modelling · Sialopentasaccharide receptor · Per residue interactions · Molecular dynamics simulations

N. Nunthaboot
Department of Chemistry, Faculty of Science,
Mahasarakham University, Mahasarakham 44150, Thailand

T. Rungrotmongkol · M. Malaisree · P. Decha · N. Kaiyawet · P. Intharathep · P. Sompornpisut · S. Hannongbua (✉)
Computational Chemistry Unit Cell, Department of Chemistry,
Faculty of Science, Chulalongkorn University,
Bangkok 10330, Thailand
e-mail: supot.h@chula.ac.th

T. Rungrotmongkol
Center of Innovative Nanotechnology,
Chulalongkorn University, Bangkok 10330, Thailand

Y. Poovorawan
Center of Excellence in Clinical Virology,
Faculty of Medicine, Chulalongkorn University,
Bangkok 10330, Thailand

S. Hannongbua
Center of Excellence for Petroleum, Petrochemicals,
and Advanced Materials, Chulalongkorn University,
Bangkok 10330, Thailand

Introduction

Since the first identification of the novel A (H1N1) influenza virus in April 2009, the outbreak of this virus has rapidly spread and encircled over 100 countries worldwide, causing more than 3,000 human deaths (April–September 2009) [1]. The World Health Organization (WHO) announced a worldwide pandemic alert level at phase 6, indicating that a global human pandemic of this virus isolate is under way [1–3]. In the primary step of the viral replication cycle, influenza infection is initiated by the viral surface homotrimeric glycoprotein hemagglutinin (HA) binding to the host membrane sialylated glycans, which act as cell receptors. Understanding of this attachment and interaction can provide a basic knowledge of how the emerging virus infects humans and is thus the main goal of this study.

Hemagglutinin is an important target for the development of both vaccines and antiviral drugs against influenza viruses. Each monomer of the homotrimer is composed of two subunits, HA1 and HA2. Whilst HA1 is known to be responsible for the viral attachment to host cell, HA2 is associated with the release of the viral RNA complexed with the RNA polymerase through membrane fusion [4–7], and thus HA is essential to both host cell targeting and cell

entry (infection). HA1 binds to host cell membrane receptors, glycans containing the terminal sialic acid which are attached to surface membrane proteins or lipids [6, 8, 9]. The specific topology, determined principally but not exclusively by the specific linkage of the terminal sialic acid to the galactose subunit and the glycan chain length, identifies the species and tissue specificity and avidity of binding, and thus its infectability and transmission rates [10]. The avian influenza virus preferentially recognizes the sialic acid α-2,3-galactose (SIA-α-2,3-GAL) linkage with a long glycan chain and cone-like topology, whilst the adopted sialic acid α-2,6-galactose (SIA-α-2,6-GAL) linkage is more favorable for both human and swine influenza viruses with longer glycan chains and an umbrella topology [10–14]. It is supposed that the alternation in host specificity of sialic acid linked to galactose from α-2,3- to α-2,6-linkage is a major barrier for influenza viruses to cross species barriers and adapt to a new host [7, 10, 15–18].

From the available information, it is clear that the binding domain of HA with the glycan receptors comprises several key structural components including the 190-helix, 130- and 220-loop domains, and several other conserved residues that give species and tissue specificity [9]. However, how this is derived is not clear and to date, the H1N1-2009 HA structures, either as free-form or receptor-bound conformation, have not yet been experimentally solved. Recently, a theoretically modeled structure of the HA–receptor complex has been published [19]. However, it represents a static view of protein–receptor interactions without dynamic capture of time-dependent properties. Therefore, in the present study, molecular dynamics (MD) simulations were performed on the homology modeled structure of the novel H1N1 HA complexed with the SIA-2,6-GAL sialopentasaccharide, a human preferential receptor, to investigate the fundamental structural characteristics, the role of conserved binding residues, and receptor binding specificity. Extensive analysis was focused on the structural properties and, in particular, on the enzyme–receptor interactions in terms of hydrogen bonding and per residue–receptor interactions.

Results and discussion

MD simulation of the novel H1N1 HA complexed with the SIA-2,6-GAL sialopentasaccharide, a human preferential receptor, was carried out over a period of 4 ns. In the last 2.5-ns simulation, the whole system is fairly stable as indicated by the small magnitude of root mean square deviation (RMSD) fluctuation of ca. 0.5 Å (Fig. 1). The simulation run could thus provide a suitable basis for the subsequent analyses.

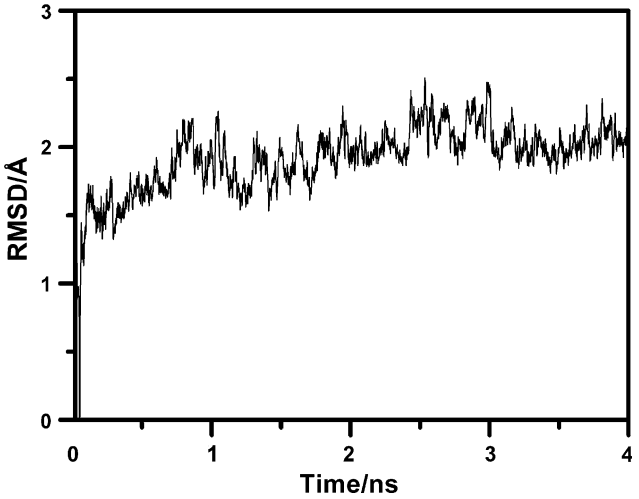

Fig. 1 Root mean square deviation (*RMSD*) of all heavy atoms of hemagglutinin and human SIA-α-2,6-GAL pentasaccharide receptor to the starting structure as a function of simulation time

The obtained human SIA-2,6-GAL sialopentasaccharide receptor was found to properly occupy the binding pocket of the 2009 H1N1 hemagglutinin, similar to what has been observed experimentally in the other viral influenza HA strains [21, 28–30], where the potentially important contact residues of the 130-loop (K133a, N133, V135, T136, and A137), 190-helix (H183, D190, and S193), and 220-loop (K222, D225, Q226, and E227) as well as Y95 (see Fig. 2a for residue positions) were revealed. Structural properties, hydrogen bonds, and per residue–receptor interactions are extensively discussed in the following sections.

Sialopentasaccharide receptor conformation

To investigate the conformational character of the human SIA-2,6-GAL sialopentasaccharide receptor, the distribution of eight important torsion angles, defined in Fig. 3a, from (1) *τ1–τ4* bridging between the saccharide units and (2) *τ5–τ8* of the functional groups of the terminal sialic acid, were measured and plotted in Fig. 3b and c, respectively.

It can clearly be seen in Fig. 3b that the *τ1* and *τ2* torsions of the first three saccharide units (SIA1, GAL2, and NAG3) show a single preferential and sharp peak, suggesting the high stability of these units which were well oriented and occupied in the binding pocket of the enzyme (Fig. 2a, b) and, therefore, that many hydrogen bonds with the HA residues were firmly formed (Fig. 4a, discussed later). The most probable glycosidic torsion angle (*τ1*, black line in Fig. 3b) was found at ca. −68° indicating the adopted *cis*-conformation of the α-2,6-linked terminal sialic acid (SIA1) to the galactose (GAL2) of the receptor. This proposed conformation is consistent with what has

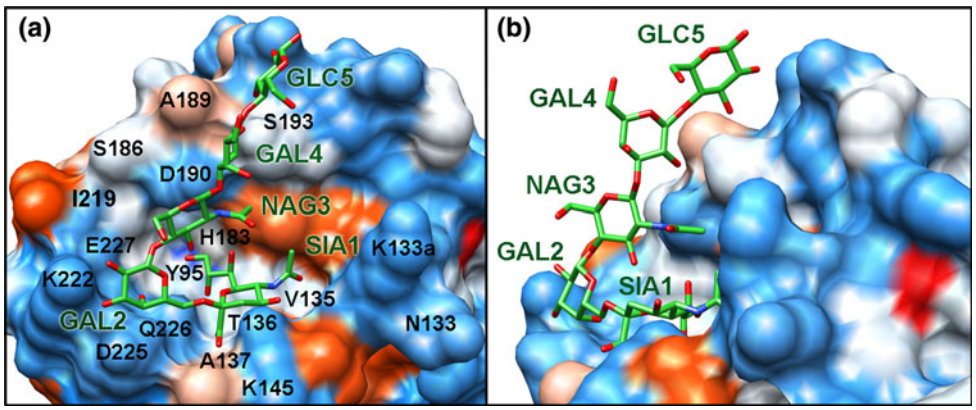

Fig. 2 **a** Top and **b** side views of the human SIA-2,6-GAL sialopentasaccharide receptor bound to the binding pocket of the 2009 H1N1 influenza HA. The potential contact residues and five units of the receptor (SIA1, GAL2, NAG3, GAL4, and GLC5) are labeled. Residue K133a is an inserted amino acid specific to the 2009 H1N1 HA. *Blue* and *orange* surfaces indicate the hydrophilic and hydrophobic features, respectively (color figure online)

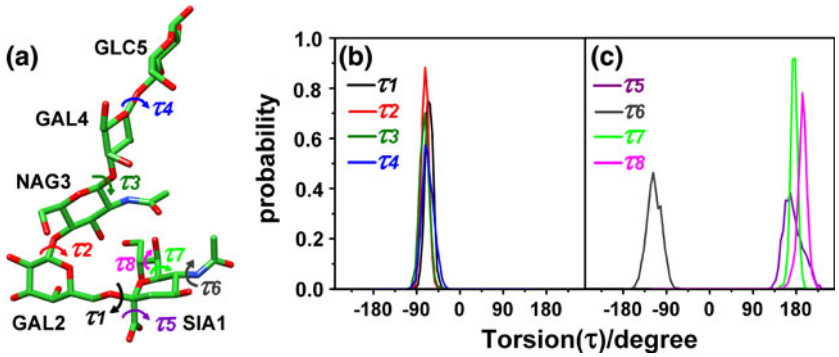

Fig. 3 **a** Definition of torsion angles of the human SIA-2, 6-GAL sialopentasaccharide receptor. Probability distributions of the **b** torsion angles ($\tau 1$–$\tau 4$) linking between each saccharide unit and **c** torsion angles ($\tau 5$–$\tau 8$) of the functional groups of the terminal sialic acid

been observed both experimentally and theoretically for the human SIA-2,6-GAL receptor binding to the influenza HA subtypes H1, H3, and H5, whose glycosidic torsion angles were observed to fall within the range of between −50° and −70° [21, 30–33]. In the same fashion, the $\tau 3$ and $\tau 4$ angles linking between the last three saccharides (NAG3, GAL4, and GLC5) showed the single preferential sharp peak at ca. −73° (Fig. 3b) indicating their high rigidity throughout the simulation period.

To reveal the conformational change of the terminal sialic acid SIA1, the torsion angles of its functional groups were further evaluated and the results are shown in Fig. 3c. Amongst the four angles, $\tau 5$ and $\tau 6$ are slightly broader than the other two angles, $\tau 7$ and $\tau 8$. This indicates that the –COO– and –NHAc groups could feasibly rotate rather than the hydrophilic group.

Enzyme–receptor hydrogen bonds

To determine the protein–receptor interactions, hydrogen bonding between the HA residues and the human SIA-α-2,6-GAL sialopentasaccharide receptor were calculated according to the two criteria: (1) a proton donor (D) and acceptor (A) distance of 3.5 Å or less and (2) a D–H···A angle of 120° or more.

The number and percentage of hydrogen bond occupation of each of the 2009 HA binding residues and all five saccharides of the receptor were evaluated, and the results are shown in Fig. 4a (see description in Table 1). At the terminal sialic acid (SIA1, see Fig. 2a), extensive interactions were found with Y95 and the highly conserved residues of the 130-loop (V135, T136, and A137), 190-helix (H183), and 220-loop (Q226). The hydroxyl oxygen of the hydrophilic group forms a strong hydrogen bond to the phenyl group of Y95. Three strong hydrogen bonds were detected between the terminal sialic acid –COO^- group and the three HA residues, T136 and A137 in the 130-loop and Q226 in the 220-loop, whilst the –NHAc moiety established two strong hydrogen bonds with the backbone nitrogen and oxygen atoms of residue V135 in the 130-loop. In addition, the hydroxyl oxygen atoms of hydrophilic side chain form strong and moderate hydrogen bonds with the imidazole ring of H183 in the 190-helix and the amide group of Q226 in the 220-loop, respectively.

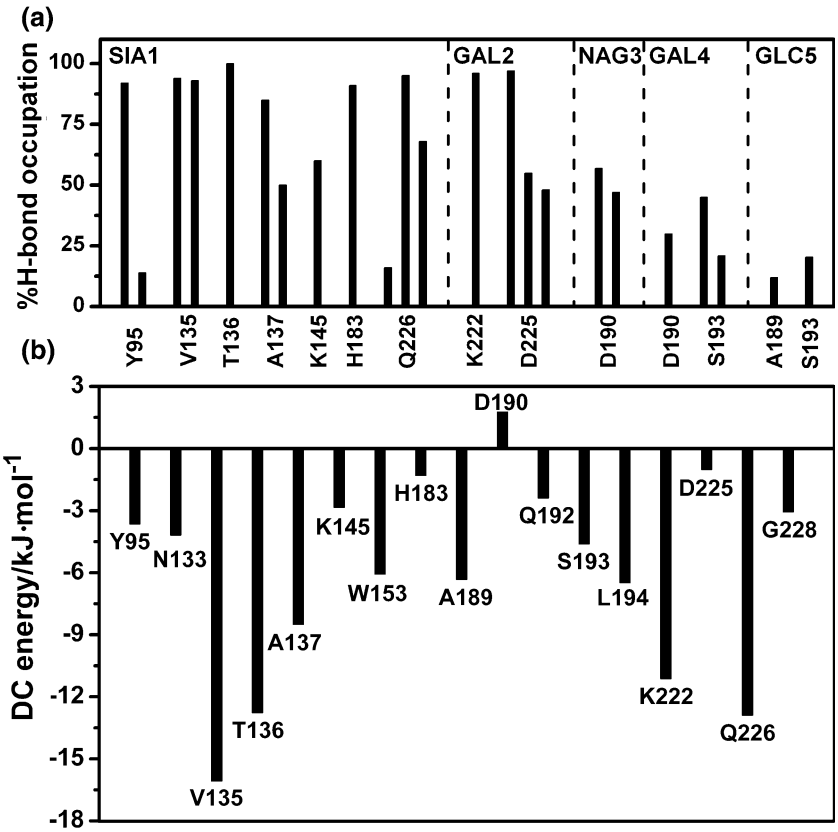

Fig. 4 **a** Hydrogen bonding occupation and **b** decomposition (*DC*) energy in kJ mol^{-1} of the individual residues of the 2009 H1N1 HA towards the human SIA-2,6-GAL sialopentasaccharide receptor (see Fig. 2 for residue labels)

Based on the numbers of hydrogen bonds (see Fig. 4a), the 130-loop is more likely to be in contact with SIA1 than the 190-helix and 220-loop, which is comparable to that of the other hemagglutins complexed with the human receptor [21, 30, 33].

For the second unit of the human SIA-2,6-GAL sialopentasaccharide receptor (GAL2), two strong hydrogen bonds were formed with the ammonium group of K222 and the backbone oxygen of D225. These hydrogen bonds were also detected in the case of the H5 HA–receptor complex, but not in the H3 and H9 HA–receptor complexes [33]. Moreover, two moderate hydrogen bonding interactions between the hydroxyl moieties of this saccharide and the carboxylate group of D225 were also found. Instead, G225, as in the crystal structure of the H1 HA–receptor complex [21, 30], forms hydrogen bonds through its backbone oxygen with the GAL2 unit. Finally, considering the other three units (NAG3, GAL4, and GLC5) of the sialopentasaccharide, they were all found to establish medium to rather weak hydrogen bond networks to the two 190-helix residues, D190 and S193, which are in agreement with the published results of the swine H1–receptor structure [21]. Interestingly, they are, however, different from what has been reported for the H3, H5, and H9 HA–receptor complexes where the last three glycans explicitly interact with the 150-loop and 190-helix [33].

Taking into account all the simulation results shown above, all important hydrogen bonds between the SIA-2,6-GAL sialopentasaccharide receptor and the residues of the 130-loop, 190-helix, and 220-loop are considerably conserved and are more likely to be similar to those observed in the H1 HA–receptor complex structure [21, 30], indicating the likely reliability of the simulated structures of the human receptor bound to the pocket of the viral H1N1-2009 HA. In addition, the results also confirm the potentially important role of the 130-loop, 190-helix, and 220-loop of the viral surface HA in attaching to SIA-2,6-GAL sialopentasaccharide glycan, which is the main receptor found in human respiratory tract host cells.

Per residue HA enzyme–SIA-2,6-GAL receptor interactions

To reveal the fundamental basis of the binding between the human SIA-2,6-GAL sialopentasaccharide receptor and the influenza HA, the interaction energies between each of the individual residues and the SIA-2,6-GAL sialopentasaccharide were evaluated by using the decomposition (DC) energy module implemented in AMBER 10. The energetic contribution was averaged over a set of 100 MD snapshots, taken at every 25 ps from the last 2.5-ns simulation.

Table 1 Hydrogen bond descriptions and interactions detected between heavy atoms of the human SIA-α-2,6-GAL pentasaccharide receptor and 2009-H1N1 hemagglutinin residues

Pentasaccharide	HA	Type	Occupation (%)
SIA1	Y95	Y95_OH_H···O8_SIA1	92
	Y95	Y95_OH···H_O9_SIA1	14
	V135	V135_N_H···O5N_SIA1	94
	V135	V135_O···H_N5_SIA1	93
	T136	T136_OG1_H···O1B_SIA1	100
	A137	A137_N_H···O1A_SIA1	85
	A137	A137_N_H···O1B_SIA1	50
	K145	K145_NZ_H···O4_SIA1	60
	H183	H183_NE2···H_O9_SIA1	91
	Q226	Q226_NE2_H···O1A_SIA1	16
	Q226	Q226_NE2_H···O1B_SIA1	95
	Q226	Q226_OE1···H_O8_SIA1	68
GAL2	K222	K222_NZ_H···O3_GAL2	96
	D225	D/G225_O···H_O4_GAL2	97
	D225	D225_OD1···H_O3_GAL2	55
	D225	D225_OD2···H_O3_GAL2	48
NAG3	D190	D190_OD1···H_N2_NAG2	57
	D190	D190_OD2···H_N2_NAG2	47
GAL4	D190	D190_OD1···H_O2_GAL4	30
	S193	S193_OG···H_O2_GAL4	45
	S193	S193_OG_H···O2_GAL4	21
GLC5	A189	T189_O···H_O6_GLC5	12
	S193	S193_OG···H_O3_GLC5	20

The evaluated DC energies of the HA residues located in the binding pocket are plotted in Fig. 4b, where the per residue interaction energies are seen to vary within the range of 2 to -17 kJ mol^{-1}. The major contribution to the enzyme–receptor interactions was gained from the conserved residues which are the members of the 130- and 220-loops: V135, T136, A137, K222, and Q226. The corresponding DC energies of less than -8 kJ mol^{-1} due to these residues agree well with the hydrogen bond data discussed above (Fig. 4a) and corroborate their important role in attaching the viral coat HA to the human SIA-2,6-GAL sialopentasaccharide receptor of susceptible host cells. The higher negative values of the DC data in Fig. 4b for the remaining residues of these two loops and the 190-helix residues (except for D190) also indicate their likely responsibilities in stabilizing the human receptor–HA complex. In some contrast, and in agreement with a previous theoretical report [34], the D190 residue was found to destabilize the protein–receptor complex.

Interestingly, as determined from their DC energies, the D225 and D190 residues do not significantly improve the enzyme–receptor binding affinity, although they interact explicitly via three hydrogen bonds with the GAL2, NAG3, and GAL4 saccharides of the SIA-2,6-GAL sialopentasaccharide, respectively (as discussed above). This can then be best understood in terms of their total interactions with the neighboring residues, since the DC energy is a summation of all interactions between a central residue and its environment, including the SIA-2,6-GAL receptor and all the residues of the respective HA enzyme. In other words, the D225 and D190 hydrogen bond energies can be destabilized by their repulsions with the other residues of the HA.

Conclusions

In the present study, the three-dimensional structure of the human SIA-2,6-GAL sialopentasaccharide receptor bound to the recently detected 2009 H1N1 HA was modeled based on a homology modeling approach and consequently performed by molecular dynamic simulations. The structural properties and protein–receptor interactions, in terms of the receptor conformation, hydrogen bonds, and per residue interaction energies, were extensively discussed and compared to the binding between the human SIA-2,6-GAL sialopentasaccharide receptor and the other HAs. Basically, comparative molecular dynamics are complementary to experimental results (tissue binding, glycan microarrays, Scatchard analysis) and do not suffer the drawback of crystallographic methods in that the glycan and HA protein show considerable flexibility in conformation which is missed, by being only a single snapshot, by crystallography methods.

Conformational analysis of the human SIA-2,6-GAL sialopentasaccharide receptor orientation throughout the simulation period confirms the adopted preferential *cis*-conformation of this receptor, as indicated by the glycosidic torsion angle between the terminal sialic acid (SIA1) and the adjacent galactose (GAL2) of ca. $-68°$. The simulated model of the 2009 H1 HA bound to the human SIA-2,6-GAL sialopentasaccharide receptor showed a well-oriented conformation of the receptor in the binding pocket of the HA enzyme and lays in the conserved regions including the 130-loop, 190-helix, and 220-loop. The sialic acid forms many strong hydrogen bonds with the HA residues V135, T136, A137, H183, and Q226. Furthermore, the GAL2 unit of the receptor was found to interact with the HA K222 and D225 residues, whilst the last three glycans established hydrogen bonds with D190 and S193. Based on a per residue interaction analysis, most receptor binding residues (especially V135, T136, A137, K222, and Q226) of the viral surface HA were found to play a stabilizing role in attaching to the human SIA-2,6-GAL sialopentasaccharide receptor of the host cell.

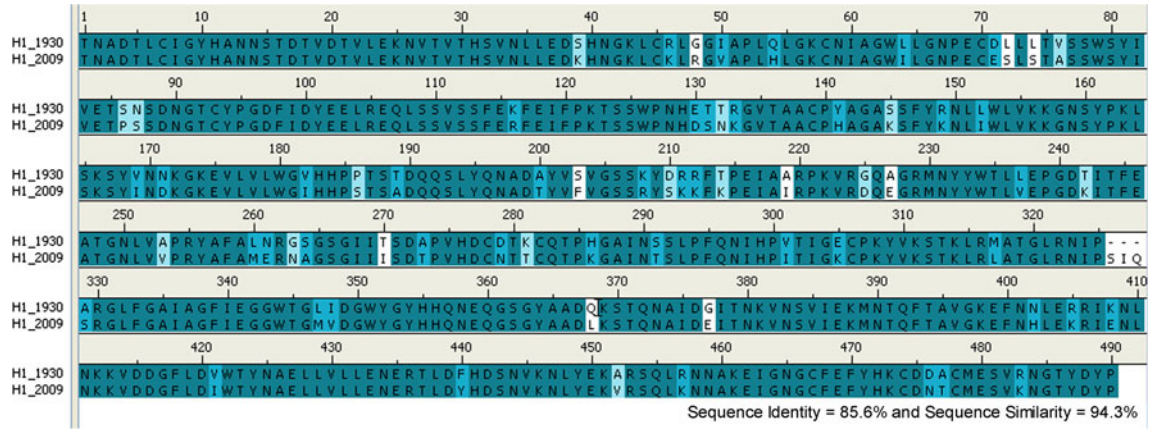

Fig. 5 Sequence alignment of 1930 and 2009 H1 hemagglutinins of influenza A (H1N1) viruses

In comparison to the other influenza HAs–human SIA-2,6-GAL sialopentasaccharide receptor complexes, the simulated results of this receptor binding to the 2009 H1N1 influenza HA provided the highest similarity to those from the structure of the H1–receptor complex. This is mainly due to the fact that they belong to the identical HA subtype and so are likely to share the highest conformational as well as primary sequence similarity. In addition, the results also show somewhat similar properties to those evaluated and observed for the H3 and H5, and H9 HAs–SIA-2,6-GAL complexes. Although many experimental aspects of the 2009 H1N1 outbreak including its virulence and pandemic potential are still uncertain, our molecular information could provide a better understanding of the first step of the viral life cycle based on how the viral surface glycoprotein HA of the 2009 influenza A (H1N1) efficiently attaches and tightly binds with the human SIA-2,6-GAL sialopentasaccharide receptor.

Materials and methods

Model of 2009 H1N1 influenza hemagglutinin complexed with human receptor

The initial structure of the 2009 H1N1 influenza HA bound with the human SIA-2,6-GAL sialopentasaccharide receptor was modeled based on the sequence which was recently isolated from children in Southern California, A/California/04/2009(H1N1) [20]. To seek the most relevant structure of the 2009 HA protein, its amino acid sequence was preliminarily aligned to all seven available crystallographic H1N1 HA structures [21]. It was found that the highest amino acid sequence similarity, at 86% identical, was with the 1930 swine H1N1 HA structure (Fig. 5). Therefore, this HA enzyme structure complexed with the human SIA-2,6-GAL sialopentasaccharide receptor (Protein Data Bank entry code 1RVT) was chosen as the template [21] for building up the HA-2009 structure by homology modeling performed by using the module implemented in Discovery Studio 2.0 [22]. The novel H1N1 HA–receptor complex was then further refined by using energy minimization and followed by multiple stepwise MD simulations.

Molecular dynamics simulations

All simulations of HA–receptor complex were carried out using the SANDER module of the AMBER 10 software package [23]. The HA protein and SIA-2,6-GAL sialopentasaccharide were parameterized by using the AMBER03 [24] and the GLYCAM06 force fields [25], respectively. All missing hydrogen atoms were added by using the LEaP module [23] and the system was subsequently solvated by a cubic box with dimensions of $66 \times 69 \times 141$ Å^3 filled with TIP3P water molecules. Normal charge states of ionizable amino acids corresponding to pH 7.0 were treated and 5 Cl^- counterions were further added to maintain neutrality on the system. A periodic boundary condition in the isobaric–isothermal (NPT) ensemble with a constant pressure of 1 atm and a temperature of 310 K was set up, whilst a Berendsen coupling time of 0.2 ps was employed to control the temperature. The SHAKE algorithm [26] was applied to constrain all hydrogen bonds using a time step of 2 fs. Non-bonded interactions were calculated with a 12-Å residue-based cutoff and the particle mesh Ewald method [27] was applied to treat the long-range electrostatic interactions. To remove unfavorable contact, the structure of the HA–receptor complexes was relaxed by performing 3,000 steps of conjugated gradient energy minimization. The whole system was subsequently heated from 0 to 310 K over 100 ps. The system was pre-equilibrated for two steps of 200-ps simulations with position restraints on the receptor atoms with the factors of 80 and 40 kJ mol^{-1} Å^{-2},

to maintain their coordinates inside the protein binding pocket. Afterwards, the complex was fully simulated for 4 ns.

Acknowledgments This work was financially supported by the Thailand Research Fund (TRF) and the Commission Higher Education (CHE). N.N. (Grant No. MRG5180298) and T.R. (Grant No. TRG5280035) acknowledge the funding for New Research from TRF. The authors are grateful for the partial support by the Rachadapisek Sompoch Endowment Fund "Emerging Health Risk Cluster", Chulalongkorn University. The Center of Excellence for Petroleum, Petrochemicals, and Advanced Materials, Chulalongkorn University, is acknowledged.

References

1. World Health Organization (2010) Global Alert and Response (GAR). http://www.who.int/csr/disease/swineflu/en/index.html. Accessed 2 May 2010
2. Zarocostas J (2009) World Health Organization declares A (H1N1) influenza pandemic. Available via http://www.bmj.com/cgi/content/extract/338/jun12_1/b2425. Accessed 2 May 2010
3. Centers for Disease Control and Prevention (2010) 2009 H1N1 flu. http://www.cdc.gov/h1n1flu/. Accessed 2 May 2010
4. Steinhauer DA (1999) Virology 258:1
5. Horimoto T, Kawaoka Y (2001) Clin Microbiol Rev 14:129
6. Cross KJ, Burleigh LM, Steinhauer DA (2001) Exp Rev Mol Med 6:1
7. Stevens J, Blixt O, Tumpey TM, Taubenberger JK, Paulson JC, Wilson IA (2006) Science 312:404
8. Wiley DC, Skehel JJ (1987) Annu Rev Biochem 56:365
9. Skehel JJ, Wiley DC (2000) Anun Rev Biochem 69:531
10. Chandrasekaran A, Srinivasan A, Raman R, Viswanathan K, Raguram S, Tumpey TM, Sasisekharan V, Sasisekharan R (2008) Nat Biotechnol 26:107
11. Bateman AC, Busch MG, Karasin AI, Bovin N, Olsen CW (2008) J Virol 82:8204
12. Gambaryan AS, Karasin AI, Tuzikov AB, Chinarev AA, Pazynina GV, Bovin NV, Matrosovich MN, Olsen CW, Klimov AI (2005) Virus Res 114:15
13. Matrosovich MN, Gambaryan AS, Teneberg S, Piskarev VE, Yamnikova SS, Lvov DK, Robertson JS, Karlsson KA (1997) Virology 233:224
14. Rogers GN, D'Souza BL (1989) Virology 173:317
15. Suzuki Y, Ito T, Suzuki T, Holl RE, Chambers TM, Kiso M, Ishida H, Kawaoka Y (2000) J Virol 74:11825
16. Russell RJ, Stevens DJ, Haire LF, Gamblin SJ, Skehel JJ (2006) Glycoconjugate J 23:85
17. van Riel D, Munster VJ, de Wit E, Rimmelzwaan GF, Fouchier RA, Osterhaus AD, Kuiken T (2007) Am J Pathol 171:1215
18. Nicholls JM, Bourne AJ, Chen H, Guan Y, Peiris JSM (2007) Respir Res 8:73
19. Soundararajan V, Tharakaraman K, Raman R, Raguram S, Shriver Z, Sasisekharan V, Sasisekharan R (2009) Nat Biotechnol 27:510
20. National Center for Biotechnology Information (NCBI) (2010) GenBank sequences from pandemic (H1N1) 2009 viruses. http://www.ncbi.nlm.nih.gov/genomes/FLU/SwineFlu.html. Accessed 2 May 2010
21. Gamblin SJ, Haire LF, Russell RJ, Stevens DJ, Xiao B, Ha Y, Vasisht N, Steinhauer DA, Daniels RS, Elliot A, Wiley DC, Skehel JJ (2004) Science 303:1838
22. Discovery Studio 2.0, Accelrys Inc., San Diego, CA, USA
23. Case DA, Darden TA, Cheatham TE, Simmerling CL, Wang J, Duke RE, Luo R, Crowley M, Walker RC, Zhang W, Merz KM, Wang B, Hayik S, Roitberg A, Seabra G, Kolossváry I, Wong KF, Paesani F, Vanicek J, Wu X, Brozell SR, Steinbrecher T, Gohlke H, Yang L, Tan C, Mongan J, Hornak V, Cui G, Mathews DH, Seetin MG, Sagui C, Babin V, Kollman PA (2008) AMBER 10, University of California, San Francisco
24. Duan Y, Wu C, Chowdhury S, Lee MC, Xiong G, Zhang W, Yang R, Cieplak P, Luo R, Lee T, Caldwell J, Wang J, Kollman P (2003) J Comput Chem 24:1999
25. Tessier MB, DeMarco ML, Yongye AB, Woods RJ (2008) Mol Simulat 34:349
26. Ryckaert JP, Ciccotti G, Berendsen HJC (1977) J Comput Phys 23:327
27. Darden T, York D, Pedersen L (1993) J Chem Phys 98:10089
28. Ha Y, Stevens DJ, Skehel JJ, Wiley DC (2001) Proc Natl Acad Sci U S A 98:11181
29. Ha Y, Stevens DJ, Skehel JJ, Wiley DC (1968) Virology 309:209
30. Lin T, Wang G, Li A, Zhang Q, Wu C, Zhang R, Cai Q, Song W, Yuen KY (2009) Virology 392:73
31. Kumari K, Gulati S, Smith DF, Gulati U, Cummings RD, Air GM (2007) Virol J 4:42
32. Li MY, Wang BH (2006) Biochem Biophys Res Commun 347:662
33. Xu D, Newhouse EI, Amaro RE, Pao HC, Cheng LS, Markwick PRL, McCammon JA, Li WW, Arzberger PW (2009) J Mol Biol 387:465
34. Iwata T, Fukuzawa K, Nakajima K, Aida-Hyugaji S, Mochizuki Y, Watanabe H, Tanaka S (2008) Comput Biol Chem 32:198