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Why does avian influenza A virus hemagglutinin bind to avian receptor stronger than to human receptor? Ab initio fragment molecular orbital studies

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Abstract

Influenza A viruses attach to α-sialosides on the target cell surface by their hemagglutinins, which strictly recognize the difference in sialic acid-galactose linkage. Why does avian virus H3 subtype bind to avian receptor Neu5Ac(α2–3)Gal stronger than to human receptor Neu5Ac(α2–6)Gal? Why does avian H3 mutated Gln226 to Leu preferentially bind to human receptor? In this paper, we theoretically answer the questions by molecular mechanics and ab initio fragment molecular orbital (FMO) calculations. The binding energy between avian H3 and avian receptor is 8.2 kcal/mol larger than that of the avian H3-human receptor complex estimated at the FMO-HF/STO-3G level, which is a reason that avian H3 binds to avian receptor stronger than to human receptor. Avian Leu226 H3 clashes to Gal unit on the avian receptor to quite decrease its binding affinity. In contrast, Gal unit on the human receptor forms intermolecular hydrophobic interaction with avian Leu226 H3 to afford moderate binding affinity.

Keywords: Influenza; Hemagglutinin; Neu5Ac-Gal; Ab initio FMO; Binding energy; Binding affinity; Computational chemistry

We have always paid attention to pandemic influenza. The new-type virus has appeared once in several decades and caused fatal pandemics such as Spanish flu (H1N1, 1918), Asian flu (H2N2, 1957), and Hong Kong Flu (H3N2, 1968). It is thought that the evolution of influenza viruses occurs in avian hosts and the new-type virus will quickly spread world-widely by annual moving of migratory bird. Recently, some clinical cases were reported that the avian influenza A/H5N1 virus infected to the human [1]. These original avian viruses do not cause human to human infection. However, if the secondary host-range mutation occurred, the new virus causes serious human outbreak because of lacking immunological experience. Unfortunately, we have already experienced the outbreak of pandemic human influenza A/H3N2 in 1968–1969.

Recent studies revealed the molecular biological bases of infection and also the mechanism of host-range alteration from avian to human. Influenza viruses attach to the specific α-sialosides on the target cell surface by their hemagglutinins:HA [2]. HA strictly recognizes the difference in sialic acid-galactose linkage and the sialic acid species. Rogers et al. have reported that influenza A virus HA Gln226 H3 subtype binds to avian receptor Neu5Ac(α2–3)Gal

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and Leu226 H3 binds to human receptor Neu5Ac (α2–6)Gal [3]. Host range of influenza A virus is changed by mutations of one or two amino acid residues at surface binding site in HA [4]. However, there is no theoretical explanation for the relationship between the binding affinity and the point mutation at 226. Why does avian H3 bind to avian receptor stronger than to human receptor? Why does avian H3 mutated Gln226 to Leu bind to human receptor stronger than to avian receptor? We report here ab initio fragment molecular orbital (FMO) studies for the interactions of avian influenza A virus Gln or Leu 226 H3 with Neu5Ac(α2–3 or α2–6)Gal receptors. We compared the interaction patterns between avian H3s and Neu5Ac-Gal receptors by the estimation of their interaction energies between the receptors and amino acid residues on the binding site of avian H3s. We also compared the binding energies between the receptors and H3s at the HF/STO-3G level by ab initio FMO methods [5] to discuss its binding affinities.

Avian H3-receptor complexes for our theoretical studies were prepared from the crystal structures of avian H3 complexed with Neu5Ac(α2–3/6)Gal analogues (pdb ID: 1MQM, 1MQN) [6]. We optimized the complexes (1470 amino acid residues, 23639 atoms) by molecular mechanics energy calculation with the CFF force field [7] and cut out the surface binding sites from the optimum complexes (70 amino acid residues, 1118 atoms) to obtain the interaction sites for ab initio FMO calculations (Fig. 1A and B). We mutated in silico Gln226 to Leu in the avian H3-receptor complexes, and set the receptor conformations to the reported orientations referring to the crystal structures of human Leu226 H3 complexed with Neu5Ac(α2–3/6)Gal [8]. In the avian Leu226 H3- Neu5Ac(α2–3)Gal complex, Leu226 clashes to Gal unit on Neu5Ac(α2–3)Gal (Fig. 1C), and suitable geometry structures were not found. In contrast, the interacting site in the avian Leu226 H3-Neu5Ac(α2–6)Gal complex was prepared by the same method without problem (Fig. 1D). Single point energies of the interaction sites A, B, and D were computed at the FMO-HF/STO-3G level. The binding sites of H3s were divided into one amino acid residue as a single fragment. Neu5Ac-Gal receptors were treated as a single fragment. The receptors and H3 binding sites were charged to −1 and +1, respectively. We computed single point energies of the interaction sites (E_{complex}), Neu5Ac-Gal receptors (E_{receptor}), and H3 binding sites (E_{H3}) at the FMO-HF/STO-3G level to estimate binding energies (ΔE) between the receptor and H3 to the following expression; $\Delta E = (E_{\text{receptor}} + E_{\text{H3}}) - E_{\text{complex}}$. Molecular mechanics calculations were carried out using Discovery Studio 1.5.1 program (Accelrys Inc.). Ab initio FMO calculations were performed by using ABINIT-MP ver. 2.0 beta program [5].

Figs. 2–4 show the interaction patterns between Neu5Ac(α2–3/6)Gal and the binding site of avian Gln/Leu 226 H3. The interaction energies of the Neu5Ac-Gal receptor with amino acid residues on the binding site at FMO-HF/STO-3G are summarized in Tables 1 and 2. The binding energies ΔE between avian H3s and avian Neu5Ac(α2–3/6)Gal receptors at the FMO-HF/STO-3G level are indicated in Table 3.

Avian Gln226 H3 theoretically interacts with avian receptor Neu5Ac(α2–3)Gal stronger than to human receptor Neu5Ac(α2–6)Gal. In the Gln226 H3-Neu5Ac(α2–3)Gal complex (Fig. 2A), Gln226 makes intermolecular hydrogen bonds with 8-OH, 1-COO on Neu5Ac and 4-OH on Gal, to give the bonding interaction energy between Neu5Ac(α2–3)Gal and Gln226 by 8.8 kcal/mol at the FMO-HF/STO-3G level (Table 1, entry 3), in addition, the glycoside oxygen O3 on Gal weekly interacts with side chain on Gln226 by 2.82 Å. This interaction network causes hydrogen bond formations of 1-COO with Ser136 and Ser137 stronger than that in the Gln226 H3-Neu5Ac(α2–6)Gal complex (Fig. 3 and Table 1: entries 6

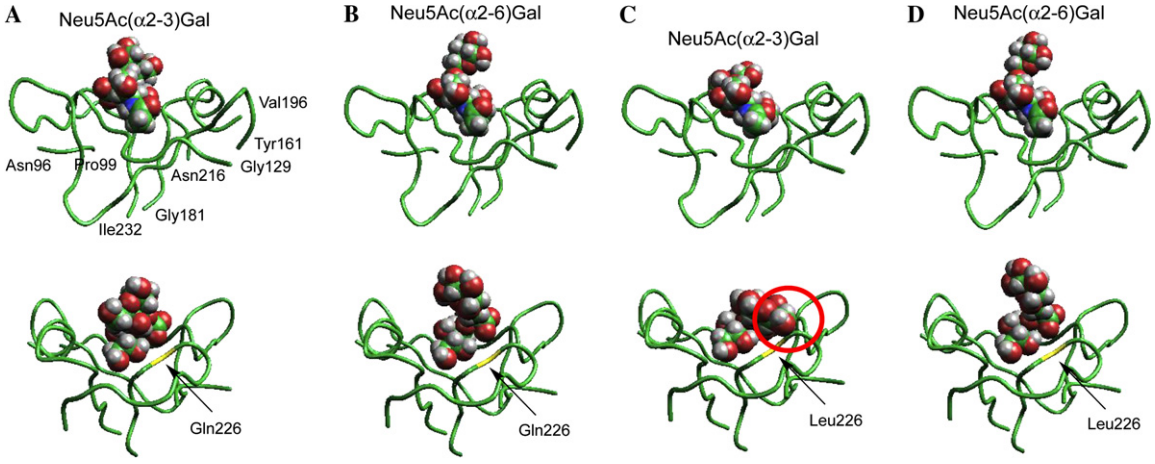

Fig. 1. Interaction sites in avian Gln/Leu 226 H3 complexed with Neu5Ac(α2–3/6)Gal receptor. (A) Gln226 H3-Neu5Ac(α2–3)Gal complex. (B) Gln226 H3-Neu5Ac(α2–6)Gal complex. (C) Leu226 H3-Neu5Ac(α2–3)Gal complex. (D) Leu226 H3-Neu5Ac(α2–6)Gal complex. The interaction sites A, B, and D were optimized by molecular mechanics energy calculations. The bottom figures are back views of the corresponding interaction sites. In the complex C, Leu226 clashes to Gal unit on Neu5Ac(α2–3)Gal at the red circle. Gln/Leu 226 are located at the yellow position as shown in the bottom figures.

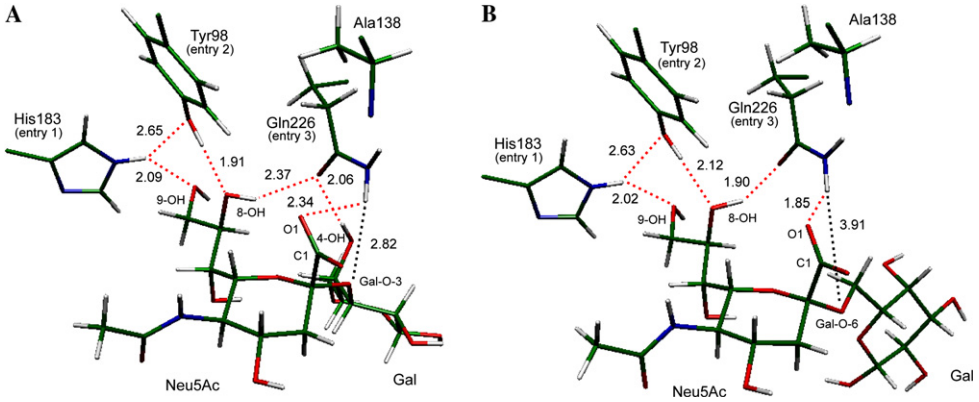

Fig. 2. Interactions of Neu5Ac(α2–3/6)Gal with amino acid residues on the binding site of avian Gln226 H3. (A) Gln226 H3-Neu5Ac(α2–3)Gal complex. (B) Gln226 H3-Neu5Ac(α2–6)Gal complex. The red and black dotted lines represent hydrogen bonds and long range interactions, respectively. Atomic distances are given in angstrom (Å). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this paper.)

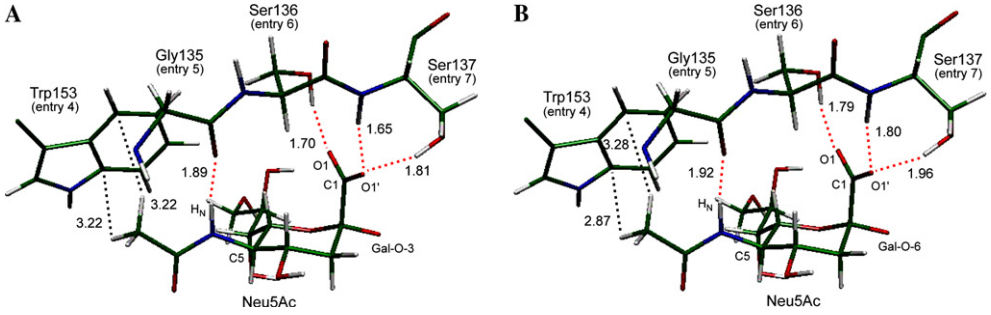

Fig. 3. Interactions of Neu5Ac on Neu5Ac(α2–3/6)Gal with amino acid residues on the binding site of avian Gln226 H3. (A) Gln226 H3-Neu5Ac(α2–3)Gal complex. (B) Gln226 H3-Neu5Ac(α2–6)Gal complex.

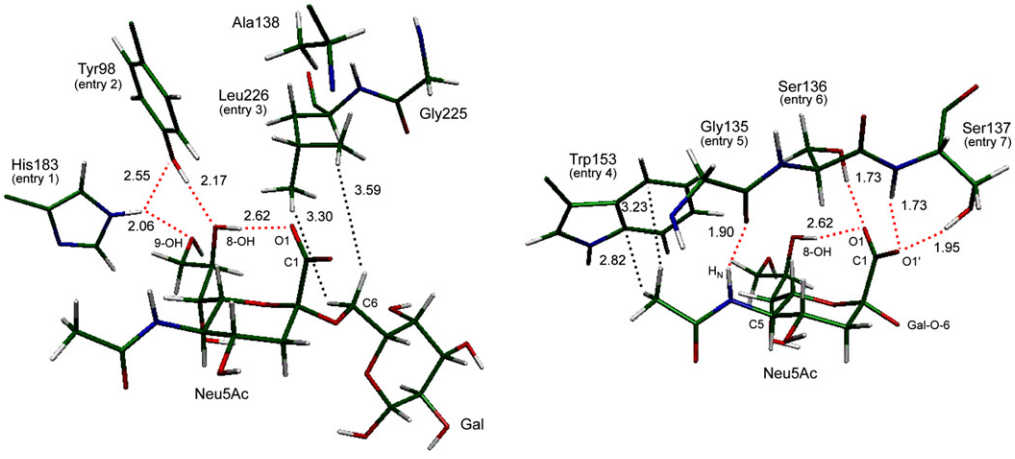

Fig. 4. Interactions of Neu5Ac(α2–6)Gal with amino acid residues on the binding site of avian Leu226 H3.

and 7). In the Neu5Ac(α2–6)Gal complex (Fig. 2B), Gln226 interacts only with 8-OH, 1-COO on Neu5Ac. Besides, the glycoside oxygen O6 on Gal makes a longer range interaction with the amide proton on Gln226, which is 1.09 Å longer than the distance of Gal-O3$\cdots$$H_2$NOC-Gln226 in the Neu5Ac(α2–3)Gal complex. The binding energy ΔE between avian Gln226 H3 and avian receptor Neu5Ac(α2–3)Gal is 8.2 kcal/mol larger than that of the avian Gln226 H3 complexed with human receptor Neu5Ac(α2–6)Gal at the FMO-HF/STO-3G level, which suggests that avian H3 binds to avian receptor stronger than to human receptor (Table 3).

In the avian Leu226 H3 complexed with avian receptor Neu5Ac(α2–3)Gal (Fig.1C), Leu226 clashes to Gal unit on

Table 1
Interaction energies of Neu5Ac(α2–3/6)Gal with amino acid residues on the binding site of avian Gln226 H3 at the FMO-HF/STO-3G level

Entry	Avian Gln226 H3 amino acid	Interaction sites on Neu5Ac-Gal		Interaction energy (kcal/mol)	
		α2–3	α2–6	α2–3	α2–6
1	His183	Neu 8,9-OH		5.2	7.5
2	Tyr98	Neu 8-OH		6.7	5.1
3	Gln226	Neu 8-OH, 1-CO1O1′ Gal 4-OH	Neu 8-OH, 1-CO1O1′	8.8	10.5
4	Trp153	Neu 5-NHCOCH$_3$		0.1	−0.4
5	Gly135	Neu 5-NHCOCH$_3$		1.9	2.7
6	Ser136	Neu 1-CO1O1′		20.5	16.2
7	Ser137	Neu 1-CO1O1′		36.5	27.7

Table 2
Interaction energies of Neu5Ac(α2–6)Gal with amino acid residues on the binding site of avian Leu226 H3 at the FMO-HF/STO-3G level

Entry	Avian Leu226 H3 amino acid	Interaction sites on Neu5Ac(α2–6)Gal	Interaction energy (kcal/mol)
1	His183	Neu 8,9-OH	7.3
2	Tyr98	Neu 8-OH	5.0
3	Leu226	Gal 6-CH$_2$	−2.2
4	Trp153	Neu 5-NHCOCH$_3$	−0.4
5	Gly135	Neu 5-NHCOCH$_3$	2.6
6	Ser136	Neu 1-CO1O1′	18.8
7	Ser137	Neu 1-CO1O1′	27.6

Table 3
The binding energies ΔE in kcal/mol between avian Gln/Leu 226 H3 and Neu5Ac(α2–3/6)Gal receptor at the FMO-HF/STO-3G level

	Avian Gln226 H3	Avian Leu226 H3
Neu5Ac(α2–3)Gal	136.9	—
Neu5Ac(α2–6)Gal	128.7	118.2

Neu5Ac(α2–3)Gal, so that its binding affinity for avian receptor quite decreased. In contrast, Leu226 in the avian Leu226 H3-Neu5Ac(α2–6)Gal complex makes hydrophobic network with 6-CH$_2$ on Gal and Ala138 (Fig. 4, left). Since 8-OH on Neu5Ac forms an intramolecular hydrogen bond with 1-COO (Fig. 4, right), the binding energy between avian Leu226 H3 and human receptor Neu5Ac (α2–6) Gal is 18.7 kcal/mol smaller than that of the avian Gln226 H3 complexed with avian receptor Neu5Ac(α2–3)Gal (Table 3).

In this paper, we provide an explanation for the host-range alteration from avian influenza A/H3 virus which shows less human virulence to the high virulent virus to human by single amino acid mutation Gln226Leu using molecular mechanics and ab initio fragment molecular orbital calculations. This method may apply to prediction of infectious level of new virus and also drug design for future mutation.

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