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Ab initio fragment molecular orbital studies of influenza virus hemagglutinin–sialosaccharide complexes toward chemical clarification about the virus host range determination

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Abstract If we predict the host range of new or mutant influenza virus in advance, we are able to measure against pandemic human influenza immediately after the new virus emerges somewhere. Influenza viral hemagglutinin(HA)–sialoside receptor interaction is a target event for *in silico* chemical prediction studies about the virus host range determination. We theoretically studied avian and human influenza A virus HA H3 subtype complexed with avian or human type receptor Neu5Acα(2-3 or 2-6)Gal analogues by *ab initio* fragment molecular orbital (FMO) method at the second order Møller–Plesset (MP2)/6–31G level, which can evaluate correctly not only electrostatic interactions but also lipophilic interactions based on van der Waals dispersion force. Avian H3 bound to avian α2-3 11.4 kcal/mol stronger than to human α2-6 in the model complexes with taking account of intermolecular lipophilic interaction. A substitution at the position 226 between Gln (avian) and Leu(human) on influenza H3 HA1 has altered

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its virus host range between avian and human. In the *ab initio* FMO studies, binding energy of avian Gln226Leu H3–human α2-6 was quite similar to that in the human H3–human α2-6 complex with amino acid sequence differences at nine positions in the models. This similarity indicates that avian Gln226Leu H3 virus can infect human with the same level as human H3 virus. Opposite mutation Leu226Gln in the human H3 gave the moderate binding energies to avian α2-3 with similarity to avian H3–α2-3 complex that supported our previous virus-sialoside binding assay. *Ab initio* FMO studies have revealed the relationship between influenza H3 virus host range and H3–α(2-3 or 2-6) receptors binding. Our theoretical approach may predict the infectious level of new viruses and point out some unknown dangerous mutation positions on HA in advance.

Keywords Influenza virus · Hemagglutinin · Sialosaccharide · Virus host range · *Ab initio* FMO

Introduction

Avian H5N1 virus has a high potential for pandemic influenza. The original H5N1 avian viruses can bind to avian type receptors on human lower respiratory tract [1, 2], however, this infection mechanism does not cause pandemic human influenza. We should always take precaution against when single or double point mutations occur in H5N1 viral hemagglutinin and its higher binding affinity to human type receptor [3–6].

If we predict the host range of new or mutant influenza virus in advance, we are able to measure against pandemic human influenza immediately after the new virus emerges somewhere. Influenza virus host range is mainly determined by HA binding affinity to host cell surface receptors sialo-glycolipids and -glycoproteins terminating in sialic acid α2-3 or α2-6 galactose [7–10], therefore the HA–sialoside interaction is a target event for *in silico* chemical prediction studies about the virus host range determination. Quantum chemical calculations will provide various chemical foresights about HA–sialosaccharide interaction without treatments of unknown dangerous influenza virus mutants. We are attempting to construct HA–sialoside complex models in order to explain the HA binding specificity using H3 subtype system [11–12]. Our current interest is focused on avian virus HA–Neu5Acα(2-3/6)Gal bindings and comparison between avian HA mutant–human α2-6 interaction and the original human HA–human α2-6 interaction.

Avian influenza A virus H3 subtype has Gln226 as one of the amino acids on sialoside binding site HA1 that binds to avian type receptor *N*-acetylneuraminic acid (Neu5Ac) α2-3 galactose (Gal) oligosaccharide stronger than to human type receptor Neu5Acα(2-6)Gal [13]. We remark that avian H3 weakly and certainly recognize human receptor α2-6 [14] on TLC-virus binding assay using synthetic sialylparaglobosides or corresponding B30 derivatives [14–18] that is supported by X-ray crystallographic structure of avian H3–human α2-6 complex [19]. Thus, it is significant to analyze the relationship between avian H3 binding affinity and the corresponding avian H3–Neu5Acα(2-3 or 2-6)Gal complexes. Human H3 virus has Leu226 instead of Gln that strongly recognizes human receptor α2-6 sialosaccharide [13, 20–24]. In the human H3 subtype, point mutations on the sialoside binding site at Tyr98Phe, His183Phe, and Leu194Ala decrease their hemagglutination to human erythrocytes [25]. Human Ser193Ile H3 agglutinates α2-6 sialoside expressed erythrocytes stronger than the original human H3 [26]. In particular, a substitution from Leu226 to Gln at the position 226 in human H3 HA1 changes its binding specificity from human α2-6 to avian α2-3 [16, 27–29]. Weis *et al*. have reported X-ray crystal structure of human Leu226Gln H3 complexed with avian type receptor α2-3 sialyllactose [20].

For the purpose of theoretical clarification about the H3–sialoside binding properties, we plausibly investigate the binding affinities of H3–Neu5Acα(2-3 or 2-6)Gal complexes by *ab initio* molecular orbital studies. It is worth while researching the energy profile on all steps in the HA–sialoside interaction composed of several events: encounter the sialoside binding site with sialoside receptor, leaving needless water solvent in the interaction process, thermodynamical relaxation on HA–sialoside complex, and multivalent effect on HA–sialoside interaction. However, we just urgently request a simple and essential approach with a reasonable cost. *Ab initio* fragment molecular orbital (FMO) calculations [30–39] to evaluate the binding energies between HA and Neu5Acα(2-3/6)Gal receptors are one of the promising strategies.

Ab initio FMO method has been applied in large biochemical systems for quantum chemical analysis to molecular interaction [40–45]. In this method, HA–sialoside complex is divided into fragments, and MO calculations are carried out on each fragment and fragment pairs. The total energy on the HA–sialoside complex is obtained as a summation of the fragment energies and interfragment interaction energies (IFIEs) [30–32, 34, 46, 47].

Our previous *ab initio* FMO studies estimated the binding energies between influenza H3 and Neu5Acα(2-3/6)Gal receptors at the FMO-Hartree-Fock(HF)/STO–3G level, and analyzed IFIEs between Neu5Ac-Gal receptor and amino acid residues on the sialoside binding site to confirm qualitatively avian H3–avian α2-3 binding affinity based on electrostatic interactions [11]. This approach would be valid qualitatively, but the FMO-HF/STO–3G method cannot evaluate significantly weak interactions such as intermolecular lipophilic stabilization which play

important roles in biochemical systems. Taking account of electron correlation effects is necessary for more quantitative treatment of these stabilizations based on van der Waals dispersion force. Recently, the FMO method has been extended to correlated calculations, which can be performed at the MP2 method [36, 48]. Including electron correlation effect, we applied the FMO-MP2 method to HA–sialoside model complexes and analyzed the hydrophilic and lipophilic interactions between Neu5Ac-Gal and amino acid residues on the sialoside binding site HA1 using IFIEs.

Methods

Energy minimized structures of avian H3 complexed with Neu5Acα(2-3 or 2-6)Gal disaccharide analogues were prepared from the corresponding X-ray crystallographic structures [19] by molecular mechanics (MM) energy calculation with the CFF force field [49] using Discovery Studio 1.5.1 program as shown in our previous works[11, 12]. We mutated *in silico* avian H3 Gln226 to Leu in the avian H3–human α2-6 disaccharide complex, changed α2-6 bond dihedral angle to human Leu226 H3 type orientation referring the crystal structure of Neu5Acα(2-6)Galβ(1-4)GlcNAcβ(1-3)Galβ(1-4)Glc: LSTc complexed with human H3 [50], and optimized its geometry by MM calculation to make the energy minimum avian Gln226Leu H3–human α2-6 complex. Optimum human H3–human α2-6 complex was given *in silico* by modification of crystallographic structure of human H3–Neu5Acα(2-3)Galβ(1-4)Glc complex [51]. We replaced the α2-3 trisaccharide receptor with Neu5Acα(2-6)Gal disaccharide based on the common Neu5Ac residue and changed its α2-6 bond dihedral angle referring LSTc conformation complexed with human H3 [50] that was similar manner to the previous study [12]. Human Leu226Gln H3–avian α2-3 complex was prepared by *in silico* point mutation from Leu226 to Gln in the human H3–avian α2-3 complex [51], changed α2-3 bond dihedral angle to avian Gln226 H3 type orientation [19] referring the crystal structure of human Leu226Gln–avian α2-3 complex [20] followed by geometry optimization. MM calculations were carried out *in vacuo* with structural determined water molecules in the crystal structures.

We cut out the H3–sialoside models (HA: N96–P99, G129–Y161, G181–V196, and N216–I232, 70 amino acid residues) from the optimum structures for *ab initio* FMO calculations (Fig. 1A). This approach covers the amino acid residues on the sialoside binding site especially position 226, besides whose range is outside of substitutions far away from the binding site. Peptide terminals in the models were treated as NH_3 and COO similar manner to the previous study [11]. Amino acid sequences of avian and human H3 differ at ten positions with same secondary structure as shown in Fig. 1B. The differences at positions 137, 145, 226, and 228 are located on the direct interaction site to Neu5Ac-Gal disaccharides. Since there are few electron density of water molecules around the sialoside binding site–Neu5Acα(2-3/6)Gal analogue complex in the H3 crystal structures, we computed the model complexes by the FMO method without water molecules.

Single point energies of the model complexes were computed at the FMO-HF level with STO–3G [11], 6–31G, and 6–31G(d) basis sets, and the correlated FMO-MP2/6–31G level of theory. The later method evaluates interfragment van der Waals type stabilization in the protein–ligand complex. Stabilizations of CH–π interaction between tryptophan and carbohydrate in the β-galactosidase-substrates or products complex were calculated at the MP2/6–31+G(d) level as 2.4–5.2 kcal/mol [52]. Stabilizing interaction energy of the fucose-benzene complex was estimated 3.0 kcal/mol at the MP2/6–31G(d,p) level of theory [53].

The 70 amino acids on the H3-sialoside complexes were divided into one amino acid residue as a single fragment using automatic fragmentation program in the ABINIT-MP package, and Neu5Ac-Gal receptors were also treated as a single fragment. The receptors and sialoside binding sites were charged to −1 and +1. We calculated single point energies of the complexes ($E_{complex}$), Neu5Ac-Gal ($E_{receptor}$),

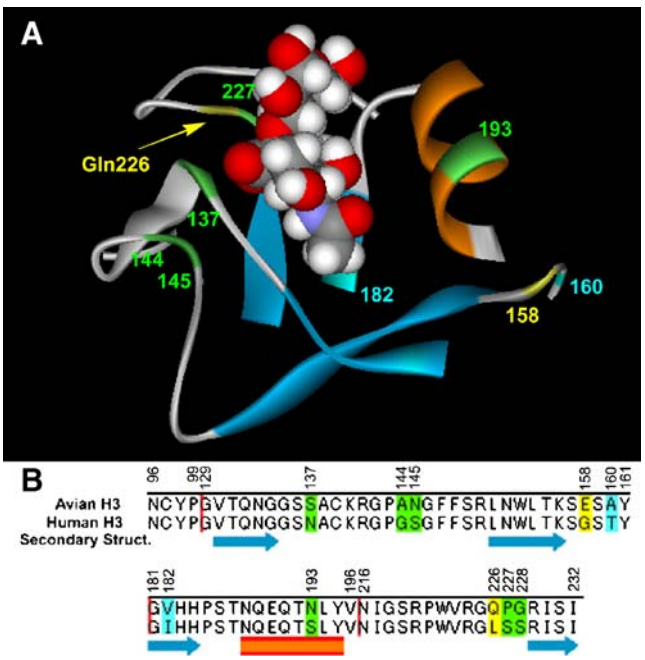

Fig. 1 Interaction site of avian influenza A virus HA H3 subtype complexed with avian type receptor Neu5Acα(2-3)Gal analogue for *ab initio* FMO studies. **A** Ribbon model; avian H3 sialoside binding site; N96–P99, G129–Y161, G181–V196, and N216–I232. CPK model; Neu5Acα(2-3)Gal disaccharide, **B** sequence alignments of avian/human H3 in the model complexes. *yellow*; non-matching residues, *green*; weak matching residues, *light blue*; strong matching residues, *red*; helix, *blue*; sheet

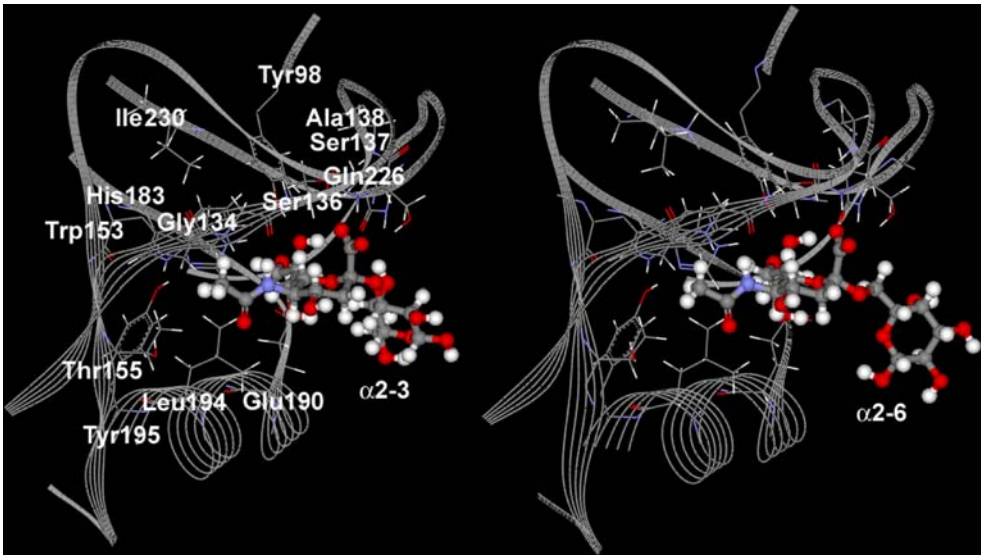

Fig. 2 Stereo depiction of the avian H3–α(2-3/6) complexes. *left*: avian H3-avian α2-3 complex. *right*: avian H3–human α2-6 complex. CPK: Neu5Acα(2-3/6)Gal disaccharides

and the binding sites (E_{H3}) to estimate binding energies (ΔE) between the receptor and H3 by the following expression; $\Delta E=(E_{receptor}+E_{H3})-E_{complex}$. *Ab initio* FMO calculations were performed by using ABINIT-MP program [30–39].

Results and discussion

Figures 2, 3, 4, and 5 show the direct interactions between Neu5Ac-Gal and sialoside binding site on H3 HA1. Binding energies ΔE and the selected IFIEs of Neu5Acα(2-3 or 2-6)Gal with amino acid residues at the FMO-HF/STO–3G and FMO-MP2/6–31G levels are summarized in Tables 1, 2, 3, 4, and 5.

ΔEs are estimated with electron correlation at the FMO-MP2/6–31G level (Table 1). In the avian H3 complexes, MP2/6–31G ΔEs are 43.5 and 40.3 kcal/mol larger than the corresponding HF/STO–3G energies to give $\Delta E_{\alpha 2\text{-}3}-\Delta E_{\alpha 2\text{-}6}$ 11.4 kcal/mol (entries 1, 3). Extension from minimal basis sets to valence double zeta basis sets at the HF level affords

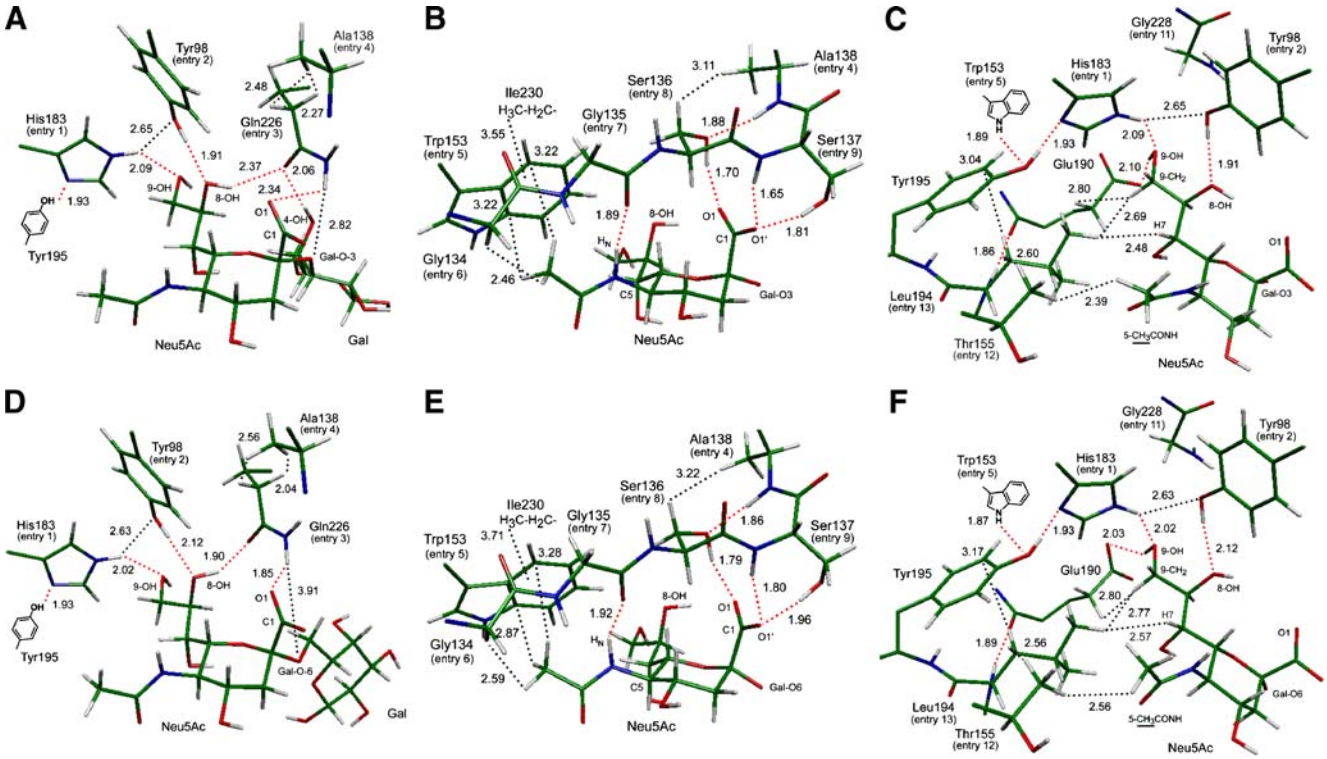

Fig. 3 Intermolecular interactions of Neu5Acα(2-3/6)Gal with amino acid residues on the sialoside binding site in avian H3. **A**–**C**; avian H3–avian α2-3 complex. **D**–**F**; avian H3–human α2-6 complex. The *red* and *black dotted lines* represent hydrogen bonds and long range interactions whose distances are given in angstrom

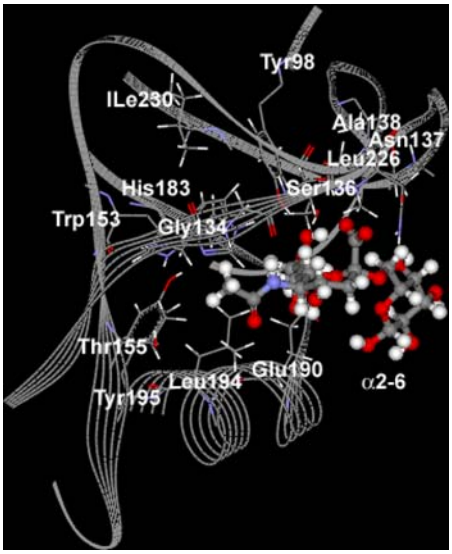

Fig. 4 Stereo depiction of the human H3–human α2-6 complex

the differences $\Delta E_{\alpha 2\text{-}3}$–$\Delta E_{\alpha 2\text{-}6}$ 13.1 kcal/mol in the avian H3–α(2-3/6) complexes.

As shown in Tables 2 and 3, various intermolecular lipophilic interactions benefit forming H3–Neu5Acα(2-3/6) Gal complexes at the FMO-MP2/6–31G level. Neu5-NHCO*CH*$_3$ makes CH–π interaction network with Trp153 and Ile230 to afford MP2/6–31G IFIEs 8.5 and 8.6 kcal/mol in avian H3–α(2-3/6) complexes, 4.0 kcal/mol in human H3–human α2-6 complex (Figs. 3B, E, and 5B, Tables 2 and 3; entry 5), while the corresponding HF/STO–3G energies do not evaluate the CH–π interaction stabilization. Gly134 and Thr155 directly interact with Neu 5-NHCO*CH*$_3$, but do not contribute to the HA–α(2-3/6) binding (Tables 2 and 3; entries 6, 12). Hydrophobic association between Leu194 and Neu7-CH, 9-CH_2 has bonding IFIEs 2.4–3.7 kcal/mol at the MP2/6–31G level (Figs. 3C, F, and 5C, Tables 2 and 3; entry 13). Intermolecular hydrophilic interactions also advantage the forming HA–sialoside complex. In the avian H3 complexes, MP2/6–31G IFIEs are 5.4–9.1 kcal/mol larger than the corresponding HF/STO–3G energies (Table 2; entries 1, 2, 4, 8, 10). Especially, Gln226 on avian H3 interacts with avian α2-3 whose MP2/6–31G IFIE is estimated 15.1 kcal/mol larger than its HF/STO–3G energy (Fig. 3A, Table 2; entry 3). In contrast, IFIE between Ser137 and Neu 1-CO1*O1'* has 33.9 kcal/mol at the MP2/6–31G which is 2.6 kcal/mol smaller than the HF/STO–3G energy (Fig. 3B, Table 2; entry 9). Gly135 clearly forms an intermolecular hydrogen bond with Neu 5-N*H*COCH$_3$, but whose IFIE are quite smaller than the others (Tables 2 and 3; entry 7).

In the following section, we discuss molecular interaction between Neu5Acα(2-3/6)Gal and amino acid residues on the sialoside binding site in each complex with Δ*E*s and IFIEs at the FMO-MP2/6–31G level.

Avian H3–Neu5Acα(2-3 or 2-6)Gal complexes

Avian H3 binds to avian receptor Neu5Acα(2-3)Gal 11.4 kcal/mol stronger than to human receptor Neu5Acα(2-6)Gal with taking account of intermolecular lipophilic stabilization at the FMO-MP2/6–31G level (Table 1; entries 1, 3). In the avian H3–avian α2-3 complex, side chain NH_2CO on Gln226 forms intermolecular hydrogen bond network with 8-OH, 1-COO on Neu5Ac, Gal 4-OH, and weakly with glycoside oxygen O3 to give IFIE 23.9 kcal/mol, which is 6.5 kcal/mol larger than the corresponding IFIE in avian H3–human α2-6 complex (Fig. 3A, D, Table 2; entry 3). Besides, hydrogen bond formations of Neu 1-COO with Ser136, Ser137 and Asn145 strongly stabilize the avian H3–avian α2-3 complex by IFIEs 27.1, 33.9, and 13.2 kcal/mol (Fig. 3B, E, Table 2; entries 8–10). The later interaction consists of intermolecular hydrogen bonds between Asn145 side chain $CONH_2$ and *O1'*O1C1 on Neu5Acα(2-3)Gal with 2.78 Å. Gly228 on influenza A virus H3 subtype correlates with the shift of virus host range between avian and human. In our study, Gly228 interacts with Neu5Acα(2-3/6)Gal disaccharides by IFIEs 4.7 and 4.3 kcal/mol (Fig. 3C, F, Table 2; entry 11).

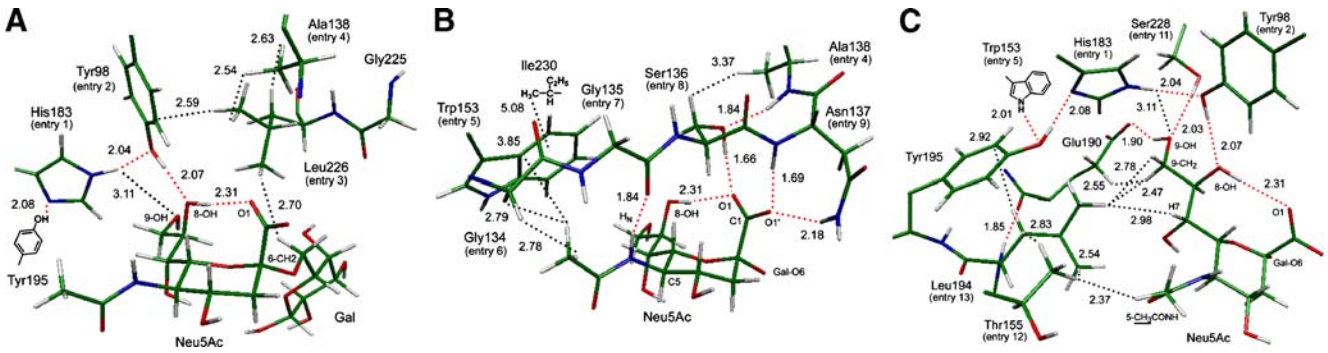

Fig. 5 Intermolecular interactions of human Neu5Acα(2-6)Gal with amino acid residues on the sialoside binding site in human H3

Table 1 Binding energies ΔE between H3s and Neu5Acα(2-3/6)Gal estimated by *ab initio* FMO calculations

[a] ΔEs were given in kilocalories per mole
[b] These data were previously reported in [11]

Entry	ΔE[a]	H3	HF/STO–3G	MP2/6–31G
1	$\Delta E_{\alpha 2-3}$	Avian	136.9[b]	180.4
2		Human Leu226Gln	137.6	179.7
3	$\Delta E_{\alpha 2-6}$	Avian	128.7[b]	169.0
4		Avian Gln226Leu	118.2[b]	157.6
5		Human	117.9	154.3

Table 2 Interfragment interaction energies of Neu5Acα(2-3/6)Gal with amino acid residues on the sialoside binding site in avian H3

Entry	Avian H3 amino acid	Interaction sites on Neu5Acα(2-3/6)Gal		α2-3		α2-6	
		α2-3	α2-6	HF/STO–3G[a]	MP2/6–31G	HF/STO–3G[a]	MP2/6–31G
1	His183	Neu 8,9-*O*H		5.2	12.4	7.5	14.5
2	Tyr98	Neu 8-*O*H		6.7	14.6	5.1	14.2
3	Gln226	Neu 8-O*H*, 1-C*O1*O1′, Gal 4-O*H*	Neu 8-O*H*, 1-C*O1*O1′	8.8	23.9	10.5	17.4
4	Ala138	Neu 1-C*O1*O1′		10.1	15.8	11.1	16.5
5	Trp153	Neu 5-NHCO*CH*$_3$		0.1	8.5	−0.4	8.6
6	Gly134	Neu 5-NHCO*CH*$_3$		−1.4	−1.3	−1.9	−1.8
7	Gly135	Neu 5-N*H*COCH$_3$		1.9	2.6	2.7	3.9
8	Ser136	Neu 1-C*O1*O1′		20.5	27.1	16.2	23.2
9	Ser137	Neu 1-CO1*O1*′		36.5	33.9	27.7	28.7
10	Asn145	Neu 1-CO1*O1*′		6.6	13.2	10.7	18.2
11	Gly228	–		1.7	4.7	1.8	4.3
12	Thr155	Neu 5-NHCO*CH*$_3$		−1.8	0.1	−2.2	−0.3
13	Leu194	Neu 7-CH, 9-CH$_2$		−1.2	3.7	−1.8	2.5
14	Sum				159.2		149.9

IFIEs were given in kilocalories per mole. IFIEs will be estimated lower by FMO-MP2/6–31G(d) energy calculations of the corresponding QM/MM optimum geometry
[a] IFIEs at the FMO-HF/STO–3G level were reported in [11]

Table 3 Interfragment interaction energies of human Neu5Acα(2-6)Gal with amino acid residues on the sialoside binding site in human H3

Entry	Human H3 amino acid	Interaction sites on Neu5Acα(2-6)Gal	HF/STO–3G	MP2/6–31G
1	His183	Neu 8,9-*O*H	3.5	8.9
2	Tyr98	Neu 8-*O*H	6.4	16.2
3	Leu226	Gal 6-CH$_2$	−1.3	6.1
4	Ala138	Neu 1-C*O1*O1′	8.6	15.7
5	Trp153	Neu 5-NHCO*CH*$_3$	−0.2	4.0
6	Gly134	Neu 5-NHCO*CH*$_3$	−1.2	−0.9
7	Gly135	Neu 5-N*H*COCH$_3$	1.0	1.1
8	Ser136	Neu 1-C*O1*O1′	24.2	29.3
9	Asn137	Neu 1-CO1*O1*′	38.5	45.5
10	Ser145	Neu 1-CO1*O1*′	0.0	2.6
11	Ser228	Neu 9-*O*H	6.8	14.8
12	Thr155	Neu 5-NHCO*CH*$_3$	−1.6	−0.3
13	Leu194	Neu 7-CH, 9-CH$_2$	−2.0	2.4
14	Sum			145.3

Table 4 Interfragment interaction energies of human Neu5Acα(2-6)Gal with amino acid residues on the sialoside binding site in avian Gln226Leu H3

Entry	Avian Q226L H3 amino acid	Interaction sites on Meu5Acα(2-6)Gal	MP2/6–31G
1	His183	Neu 8,9-*O*H	14.3
2	Tyr98	Neu 8-*O*H	14.5
3	Leu226	Gal 6-CH_2	4.8
4	Ala138	Neu 1-CO1O1′	16.5
5	Trp153	Neu 5-NHCOCH_3	8.6
6	Gly134	Neu 5-NHCOCH_3	−1.6
7	Gly135	Neu 5-N*H*COCH$_3$	3.6
8	Ser136	Neu 1-C*O1*O1′	25.0
9	Ser137	Neu 1-CO1*O1*′	26.2
10	Asn145	Neu 1-CO1*O1*′	18.1
11	Gly228	–	4.4
12	Thr155	Neu 5-NHCOCH_3	−0.2
13	Leu194	Neu 7-CH, 9-CH_2	2.7
14	Sum		136.9

Comparison between ΔEs and sums of 13 IFIEs, the formers are about 20 kcal/mol larger than the 13 IFIEs sums (Table 1; entries 1, 3, Table 2; entry 14). IFIEs sum difference of 9.3 kcal/mol mainly causes the binding energy difference ($\Delta E_{\alpha 2\text{-}3}$–$\Delta E_{\alpha 2\text{-}6}$) of 11.4 kcal/mol. In the X-ray crystal structure of avian H3 complexed with α(2-3 or 2-6)-pentasaccharide receptors, the sialoside binding site reliably recognizes the non-reducing terminal Neu5Ac-Gal [19] that approximately allows our theoretical approach.

Human H3–Human Neu5Acα(2-6)Gal complex

Binding energy ΔE in the human H3–human Neu5Acα(2-6)Gal disaccharide was estimated 154.3 kcal/mol at the FMO-MP2/6–31G level (Table 1; entry 5). Human H3 complex has an intramolecular hydrogen bond at Neu 8-OH···O1C1O1' instead of intermolecular Gln226···Neu5Ac interaction observed in the avian H3 complex (Fig. 5A), thus ΔE in the human H3 complex is smaller by 15 kcal/mol than the corresponding ΔE in the avian Gln226 H3 complex (Table 1; entries 3, 5). Neu 1-COO interacts with Ser136 by 29.3 kcal/mol, Asn137 by 45.5 kcal/mol, and Ser145 by 2.6 kcal/mol (Table 3; entries 8–10) whose summation is larger by 7.3 kcal/mol than that in the avian H3–human α2-6 complex (Table 2; entries 8–10). Intermolecular hydrogen bond network between His183, Tyr98 and Neu 9,8-OH stabilizes the human H3–human α2-6 complex with IFIEs of 8.9 and 16.2 kcal/mol (Fig. 5C, Table 3; entries 1, 2), therefore human Tyr98Phe or His183Phe H3 viruses lose this stabilization by hydrogen bonds decomposition to decrease severely their binding affinities [25]. Tyr98 forms a hydrogen bond with His183 by IFIE of 10.5 kcal/mol, and CH–π interaction with Leu226 by 1.2 kcal/mol.

Inter- and intra-molecular lipophilic association is significant for the human H3–human α2-6 binding. Leu226 interacts with Gal 6-CH_2 on human α2-6 by lipophilic IFIE of 6.1 kcal/mol (Fig. 5A, Table 3; entry 3)

Table 5 Interfragment interaction energies of avian Neu5Acα(2-3)Gal with amino acid residues on the sialoside binding site in human Leu226Gln H3

Entry	Human L226Q H3 amino acid	Interaction sites on Neu 5Acα(2-3)Gal	MP2/6–31G
1	His183	Neu 8,9-*O*H	8.6
2	Tyr98	Neu 8-*O*H	15.6
3	Gln226	Neu 8-O*H*, 1-C*O1*O1′, Gal 4-O*H*	31.3
4	Ala138	Neu 1-C*O1*O1′	15.2
5	Trp153	Neu 5-NHCOCH_3	7.9
6	Gly134	Neu 5-NHCOCH_3	−1.3
7	Gly135	Neu 5-N*H*COCH$_3$	2.6
8	Ser136	Neu 1-C*O1*O1′	27.4
9	Asn137	Neu 1-CO1*O1*′	49.1
10	Ser145	Neu 1-CO1*O1*′	3.1
11	Ser228	Neu 9-*O*H	14.3
12	Thr155	Neu 5-NHCOCH_3	−0.4
13	Leu194	Neu 7-CH, 9-CH_2	1.6
14	Sum		175.0

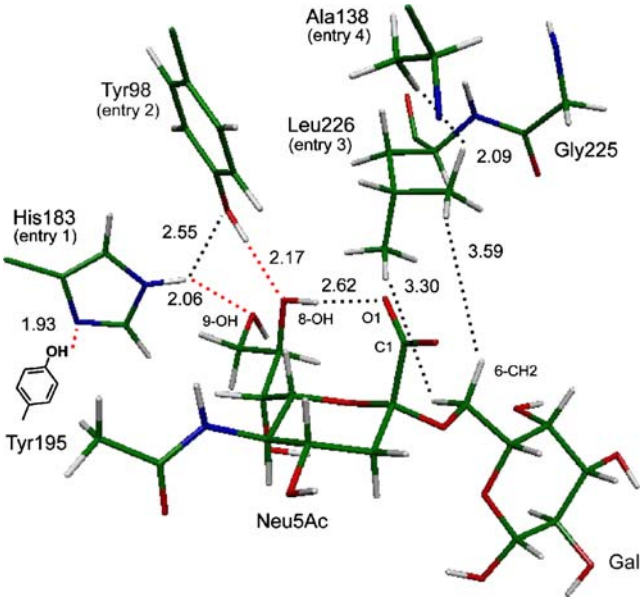

Fig. 6 Intermolecular interactions of human Neu5Acα(2-6)Gal with amino acid residues on the sialoside binding site in avian Gln226Leu H3

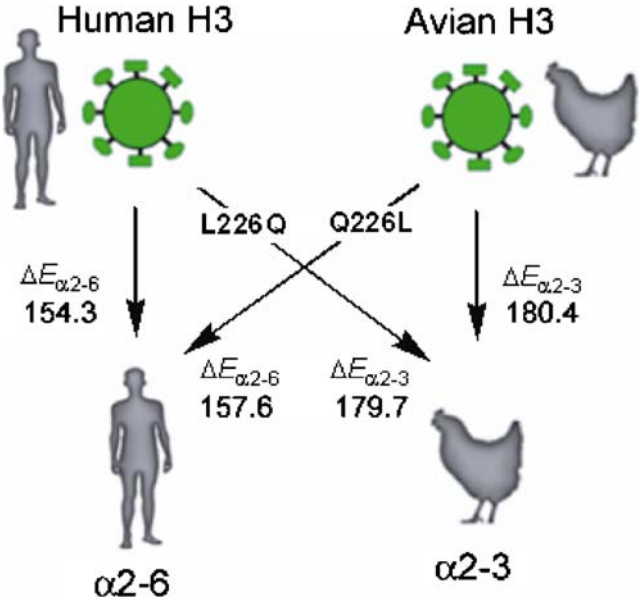

Fig. 8 Binding energy profile at the FMO-MP2/6–31G level on the relationship between influenza H3 virus host range and H3–α(2-3 or 2-6) receptors binding. Δ*E*s are shown in Table 1

that is supported by a lipophilic network beyond Leu226 consisted Tyr98, Pro99, Ala138, $(CH_2)_3$ on Arg220 and Arg229, Ile230, and Trp153. Hydrophobic site on Neu 7-CH 9-CH_2 associates with lipophilic Leu194 by IFIE of 2.4 kcal/mol (Fig. 5C, Table 3; entry 13) supported by hydrophobic groups around Leu194 such as Thr155, Tyr195, $(CH_2)_2$ on Glu190, and CH_2 on Ser193. Since human Leu194Ala H3 virus does not agglutinate human erythrocyte [25], the lipophilic association around Leu194 is significant for the human H3–human α2-6 binding affinity. A substitution at Ser193 to Ile in human H3 HA1 increases the hydrophobic capacity around Leu194 to give higher binding affinity to human α2-6 [26]. The two lipophilic networks around Leu226···Gal 6-CH_2 and Leu194···Neu 7-CH 9-CH_2 are connected by a hydrogen bond formation of indole NH Trp153 with OH Tyr195 (Fig. 5C). However, both substitutions Trp153Phe or Tyr195Phe partially decrease its binding affinity [8, 25].

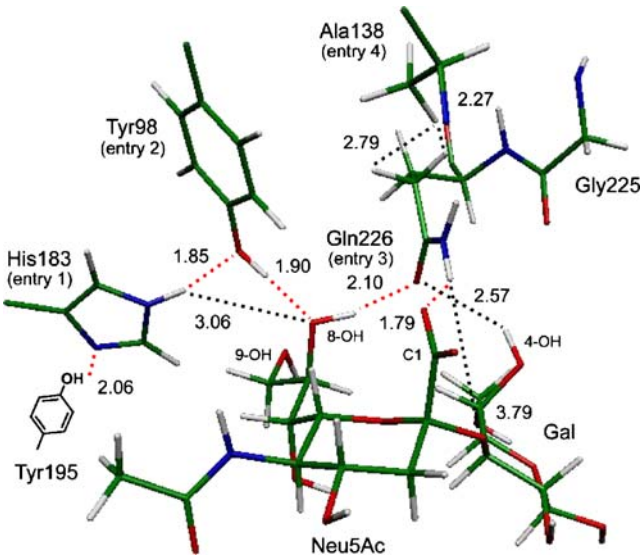

Fig. 7 Intermolecular interactions of avian Neu5Acα(2-3)Gal with amino acid residues on the sialoside binding site in human Leu226Gln H3

Avian Gln226Leu H3–Human Neu5Acα(2-6)Gal and human Leu226Gln H3–Avian Neu5Acα(2-3)Gal complexes

Figures 6 and 7 show the interaction of Neu5Ac-Gal with avian Gln226Leu/human Leu226Gln H3s. Avian Gln226Leu H3 moderately binds to human α2-6 with Δ*E* 157.6 kcal/mol at the FMO-MP2/6–31G level (Table 1; entry 4). This binding energy is quite similar to $\Delta E_{\alpha 2\text{-}6}$ 154.3 kcal/mol in the human H3 complex with amino acid difference at nine positions (Fig. 1B), therefore avian Gln226Leu H3 virus can infect human with the same level as human H3 virus. In the avian Gln226Leu H3–human α2-6 complex, Leu226 associates with lipophilic Gal 6-CH_2 whose IFIE is smaller by 1.3 kcal/mol than that of Leu226···Gal 6-CH_2 interaction in the human H3 complex (Tables 3 and 4; entry 3). Besides Neu 8-O*H* makes an intramolecular hydrogen bond with *O1*C1O1'-Neu similar manner to that in the human Leu226 H3 complex (Figs. 5A and 6).

Human Leu226Gln H3 interacts with avian α2-3 by $\Delta E_{\alpha 2\text{-}3}$ of 179.7 kcal/mol that is almost the same value as $\Delta E_{\alpha 2\text{-}3}$ of 180.4 kcal/mol in the avian H3 complex at the FMO-MP2/6–31G level (Table 1; entries 1, 2). In the human Leu226Gln H3–avian α2-3 complex, Gln226 makes intermolecular hydrogen bond network with 8-OH, 1-CO1O1' on Neu5Ac and Gal 4-OH to give IFIE 31.1 kcal/mol (Fig. 7, Table 5; entry 3). We previously reported that A/Udorn Leu226Gln (human Leu226Gln H3N2) bound to avian α2-3 receptor [14]. Our *ab initio* FMO studies have confirmed that human Leu226Gln H3 moderately interacts with avian α2-3 analogue at the correlated FMO-MP2/6–31G level.

Conclusion

We theoretically studied influenza A virus hemagglutinin H3 subtype complexed with avian/human type receptor Neu5Acα(2-3 or 2-6)Gal analogues by *ab initio* FMO method at the correlated MP2/6–31G level. Avian H3 bound to avian α2-3 11.4 kcal/mol stronger than to human α2-6 in the model complexes with taking account of intermolecular lipophilic interaction. Single point substitution at the position 226 on H3 subtype sialoside binding site HA1 changes its binding affinity between avian α2-3 and human α2-6. Our *ab initio* FMO studies showed that the binding energy of avian Gln226Leu H3 with human α2-6 was similar value to that in the human H3–human α2-6 complex at the FMO-MP2/6–31G level with amino acid differences at nine positions in our models. Thus avian Gln226Leu H3 virus can infect human with the same level as human H3 virus. Opposite mutation Leu226Gln in the human H3 gave the moderate binding energy to avian α2-3 that supported our previous virus-sialoside binding assay.

As shown in a schematic summary (Fig. 8), *ab initio* FMO studies revealed the relationship between influenza H3 virus host range and H3–α(2-3 or 2-6) receptors binding. Our theoretical approach will predict the infectious level of new viruses and point out some unknown dangerous mutation positions on HA in advance against human pandemic influenza.

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