Chemical Insight Into the Influenza A Virus Hemagglutinin Binding to the Sialoside Revealed by the Fragment Molecular Orbital Method

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Abstract: The present mini-review aims first at an introduction to two thermodynamic essentials of the binding between the influenza A virus hemagglutinin (HA) and the cell surface receptor sialoside, (1) the equilibrium 1:1 binding of the HA with the sialoside, (2) the polyvalent effect of the HA binding to the polyvalent sialoside. Second, the review introduces the fragment molecular orbital (FMO) studies of the HA-sialoside (1:1) complexes. The recent FMO method with the polarizable continuum model as one of the residue-based energy analysis method has revealed the role of key amino acid residue on the selective HA subtype H3 binding to the sialosides.

Keywords: Influenza, hemagglutinin, sialosaccharide, fragment molecular orbital, MP2, PCM.

1. INTRODUCTION

Selective binding of the influenza A virus hemagglutinin (HA) to the host cell receptor sialosaccharide concerns the viral host-range determination [1,2]. The recent biotechnology with the modern experiments, e.g. reverse genetics [3], glycan array [4,5], and sugar-chain immobiliezed gold nano particle [6], and a portable impedance biosensor [7], is almost able to monitor the binding affinities of new viruses toward the major human-type sialoside Neu5Acα2-6Gal [1,2].

However, the monitoring system does not clarify the chemical mechanisms of the follow fundamental phenomena; the strong binding between the human viral HA and the Neu5Acα2-6Gal, alteration of the HA binding affinity from the avian-type sialoside Neu5Acα2-3Gal to the Neu5Acα2-6Gal caused by the one or two-points substitutions of the amino acid residues on HA [8-13].

To understand their mechanisms, it is worth computational studying the HA-sialoside interaction on the basis of the experimental binding affinity [14-18] and the corresponding X-ray crystallographic structures. Recently, the fragment molecular orbital (FMO) method [19-22] as one of the residue-based energy analyses is very useful to find the role of key amino acid residue on the selective HA binding to sialosides. In perspectives, the FMO method collaborated with the molecular mechanics calculations, molecular dynamics simulations, and their hybrid approaches, will help us to predict the binding affinities of new viral HAs and their HA mutants to the Neu5Acα2-6Gal.

The present mini-review introduces the experimental binding between HA and sialoside, which includes the two thermodynamic essentials; (1) the equilibrium 1:1 binding of the HA with the sialoside, (2) the polyvalent effect of the HA binding to the polyvalent sialoside. Second, the review summarizes the recent FMO studies of the HA subtype H3-sialoside (1:1) complexes.

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2. BINDING AFFINITY BETWEEN THE INFLUENZA HEMAGGLUTININ AND THE SIALOSIDE

The equilibrium dissociation constant K_D between the soluble bromelain-released HA (BHA) and the sialooligosaccharides was on the order of μM~mM measured by the nuclear magnetic resonance (NMR) titration studies [14, 23], besides the order was reproduced by the fluorescence polarization titration study [24]. Therefore, the equilibrium BHA-sialoside interaction was weak as similar to the other lectin-carbohydrate interactions at the equilibrium 1:1 binding [25,26]. Generally, the weak lectin-carbohydrate interaction on the K_D $10^{-6\sim-3}$M was achieved by the balance between the fast association rate ($10^{4\sim6}$ $M^{-1}s^{-1}$) and the fast dissociation rate ($10^{0\sim2}$ s^{-1}) in the equilibrium solution [27,28].

In addition to the weak BHA-sialoside binding in equilibrium, the binding selectivity of the human viral BHA subtype H3 to the human-type sialoside Neu5Acα2-6Galβ1-4Glc was not intense so much in the equilibrium solution. As an evidence, the intrinsic K_D 2.1 ± 0.3 mM at 297 ± 1K (binding Gibbs energy ΔG_{bind} = -3.7~ -3.6 kcal/mol) of the human viral BHA with Neu5Acα2-6Galβ1-4Glc was only 1.0 mM smaller than the K_D 3.2 ± 0.6 mM of the avian-type sialoside Neu5Acα2-3Galb1-4Glc [14].

The BHA existed as a trimer of HA1-HA2 unit with the sialoside binding site laid on the top face of each HA1 domain [8, 29]. With the trivalent sialoside binding site on BHA, the equilibrium BHA-sialoside interaction approximated the simple 1:1 binding mode analyzed by the NMR titration study [14]. The simple binding mode suggested that the trivalent HA-sialoside binding was not controlled by the homotropic allosteric effect of sialoside. In other words, the

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BHA had the equivalent three binding sites for the Neu5Acα2-6Galβ1-4Glc with the first K_D 0.7 ± 0.3 mM (ΔG_{bind} -4.4~ -4.2 kcal/mol), the second K_D 2.1 ± 0.3 mM (equal to the intrinsic K_D, ΔG_{bind} -3.7~ -3.6 kcal/mol), and the third K_D 6.3 ± 0.3 mM (ΔG_{bind} -3.1~ -2.9 kcal/mol).

Both the weak HA-sialoside binding and its selectivity were very enhanced by the polyvalent effect of the sialoside receptor such as soluble sialoglycopolymers [30,31], sialoglycolipids-buried membrane [32], and sialoside-bound plate [5, 33-35]. The polyvalent effect did not depend on the polyvalency of HA because the K_D for the binding between the HA on intact virion and the monovalent sialoside was the same order with the K_D for BHA binding to the monovalent sialoside [36]. As the additional evidences, the monovalent sialoside derivatives inhibited the virus adsorption to erythrocytes with the inhibition constant K_i on the order of μM~mM [37,38] and the concentration for 50% inhibition on the order of mM [39,40].

These experimental results and discussion suggest that the HA-sialoside binding bases on the two thermodynamic essentials, (1) the equilibrium 1:1 binding of the HA with the sialoside and (2) the polyvalent effect of the HA binding to the polyvalent sialoside [41,42]. The former essence has been targeted by the all-atom computational studies involving the quantum chemical calculations, the molecular mechanics calculations, the classical molecular dynamics (MD) simulations [43-49], and their hybrid approaches. The latter will be studied by the macroscopic computational approaches [50].

3. THE FRAGMENT MOLECULAR ORBITAL STUDY OF THE INFLUENZA HEMAGGLUTININ-SIALOSIDE COMPLEX

On the basis of the X-ray crystallographic structures of various HAs in complexes with the Neu5Acα2-3 and α2-6Gal oligosaccharides, the 1:1 binding between HA and sialoside has been studied by the ab initio based FMO method. In 2006, the first trial study was performed at the FMO-Hartree-Fock (HF)/STO-3G level in the gas phase small model of HA-sialoside complexes (70 amino acid residues, about 1100 atoms) to find the key amino acid residue on the selective binding of HA subtype H3 to the sialosides [51]. In 2008, the small model complexes in gas phase were re-studied correctly by the FMO method at the second-order Møller-Plesset perturbation theory (MP2) [52,53] with the 6-31G basis sets [54]. The full HA1 domain of human viral H3 in complex with the human-type sialoside Neu5Acα2-6Gal (328 amino acid residues, 5068 atoms) was studied in gas phase at the FMO-HF/STO-3G level in 2007 [55], at the FMO-MP2/6-31G level in 2009 [56], that demanded the consideration of the backyard bulkiness beyond the sialoside binding site. In 2008, Iwata *et al*. applied first the FMO-MP2 method to the truncated model of several HA-sialoside complexes in gas phase to discuss some important interaction patterns qualitatively [57]. In 2011, Fukuzawa *et al*. applied the gas phase FMO-MP2/6-31G(d) calculations to the HAs subtype H1 in complex with the sialooligosaccharides to discuss the electrostatic residue interactions without solvation effect [58].

In 2010, the FMO method with the polarizable continuum model (PCM) [59,60] was applied to the full HA1 domain in complex with the sialoside at the MP2/6-31G(d) level [61]. The FMO-MP2/PCM approach with the molecular mechanics level normal mode analysis of the solute entropy change provided the reasonable relative ΔG_{bind} in the equilibrium 1:1 binding of the H3 with the sialoside. Moreover, the pair-interaction energy decomposition analysis [62] in the FMO framework enabled us to estimate the residue-based interaction energies of the static HA-sialoside recognition including the electrostatic interaction energy under the whole system (coulomb interaction and polarization), dispersion interaction energy, exchange-repulsion interaction energy, charge-transfer interaction energy with the higher-order mixed terms, and the de-solvation free energy *via* the complex formation.

The residue-based energy analysis revealed the role of key amino acid residue in the selective HA-sialoside binding [61]. In the detail study of the HA subtype H3 in complexes with the Neu5Acα2-3 and α2-6Gal disaccharide, the strong avian H3 (A/duck/Ukraine/1963 strain, H3N8) binding to the avian sialoside Neu5Acα2-3Gal was based on the hydrogen bond interaction between the 4-OH group on Gal residue and the side-chain $CONH_2$ on the Gln226 under the hydrogen bond network formation between the 1-COO group on Neu5Ac residue, amino acid residues at Ser136, Ser137, and Asn145. In addition, this hydrophilic interaction was supported by the surrounding hydrogen bonds, Gln226-$CONH_2$···HO-8 on Neu5Ac, Tyr98-OH···OH-8 Neu5Ac, and His183-imidazole H^{NE}···O8 and O9-Neu5Ac. The interaction manner was stable in the isobaric-isothermal (NPT-constant) classical MD simulation. The hydrophilic interaction between avian H3 and avian Neu5Acα2-3Gal largely stabilized the complex with the large desolvation energy penalty and the solute entropic penalty to give the ΔG_{bind} advantage of -6.8 kcal/mol than the ΔG_{bind} in avian H3-human Neu5Acα2-6Gal complex. These approaches with the amino acid sequence alignments of comprehensive HA [61, 63-67] provided the chemical insight into the roots of selective HA binding to the sialosides.

The reason why a substitution Gln226Leu in avian H3 altered the binding affinity from the avian Neu5Acα2-3Gal to the human Neu5Acα2-6Gal was also explained by the FMO-MP2/PCM method with the 6-31G(d) basis sets [61]. The iso-butyl group on Leu226 interacted with the Gal residue on Neu5Acα2-6Gal by utilizing the intermolecular dispersion with the small de-solvation energy penalty. Besides, the loose Leu226···Neu5Acα2-6Gal association caused the smaller entropic penalty. However, the hydrophobic Leu226···Neu5Acα2-6Gal interaction was unstable in the normal NPT-constant classical MD simulation. The Leu226···Neu5Acα2-6Gal association was achieved on the hydrophobic surface of HA, thus this interaction was exposed to the bulk solvent from the direction of Neu5Acα2-6Gal binding. With the situation, water molecules were inserted into the Leu226···Gal association under the equilibration by the NPT-constant MD simulation. When the waters were inserted once, they were never excluded from the surface hydrophobic site despite the Gibbs energy advantage of the intermolecular hydrophobic association.

The A/Aichi/2/1968 H3 (X-31, human H3N2) in complex with the human Neu5Acα2-6Gal had an intramolecular hydrogen bond at Neu5Ac 8-OH···OOC-1 Neu5Ac due to the lipophilic Leu226, thus the human H3 Leu 226·Neu5Acα2-6Gal association suffered a smaller desolvation penalty than in the hydrophilic avian H3···Gln226 avian Neu5Acα2-3Gal interaction. Unexpectedly, the FMO-MP2/PCM study suggested that the strong human H3-Neu5Acα2-6Gal binding did not depend on the additional intermolecular hydrogen bond Ser228-OH···OH-9 Neu5Ac due to suffering the large desolvation penalty *via* the hydrogen bond formation. Even if the Ser228-OH···OH-9 Neu5Ac interaction disappears, the other two hydrogen bonds Glu190-COO···HO-9 Neu5Ac and His183 imidazole $H^{N\varepsilon}$···OH-9 Neu5Ac compensate. Indeed, the substitution of Ser228Gly on the X-31 H3 HA1 retained the large human H3-human erythrocyte binding affinity [68]. Moreover, in spite of the A/Aichi/2/1968 H3-Neu5Acα2-6Gal interaction achieved by the hydrogen bond network around the 1-COO Neu5Ac with the amino acid residues Ser136, Asn137, and Ser145, this hydrophilic interaction did not contribute sufficiently to the distinctive human H3-Neu5Acα2-6Gal binding. Therefore, possible amino acid substitutions have optimized the recognition of 1-COO Neu5Ac and α2-6 bond to accelerate the strong human H3−human α2-6 binding since 1968. On the sequence alignment for the known human H3s, the Ser136 preservation is significant for the recognition of 1-COO Neu5Ac. Actually, each of the one-point substitutions Ser136Thr [68], Ser136Ala [68], Ser136Cys [69], Ser136Asn [70] decreased the human erythrocyte binding. The Asn145 and Lys145 sometimes required a hydrophobic Tyr and Phe at position 137 [61].

The NPT-constant MD simulation confirmed the validity of above static FMO-MP2/PCM study [61]. The MD simulations of the HA-sialoside complexes resulted that weakly constrained water molecules supported the interactions between active site amino acids and Neu5Ac residue from a direction of bulk solvent side regardless of Neu5Acα2-3 Gal and Neu5Ac α2-6Gal. The constrained water formed a hydrogen bond network with 9-OH group on Neu5Ac residue that was often replaced by other waters in a bulk solvent phase. These solvent properties can be qualitatively estimated by the FMO/PCM approach.

The FMO-MP2/PCM study with the molecular mechanics level harmonic normal mode analysis did not consider the full value of the solute entropic change [71-75] *via* the HA-sialoside complex formation. Nevertheless, the calculated binding energies qualitatively reproduced the available experimental order *in vitro* X-31 human virus strain H3 system [61]. Thus, the large-scale conformational effect with anharmonicity [71-75] did not qualitatively influenced the simple 1:1 binding mode of the selective binding between HA and monovalent sialoside on the basis of fast association rate and fast dissociation rate in equilibrium.

4. CONCLUSIONS

The mini-review has introduced the two thermodynamic essentials of the binding between the influenza A virus hemagglutinin (HA) and the cell surface receptor sialoside, (1) the equilibrium 1:1 binding of the HA with the sialoside, (2) the polyvalent effect of the HA binding to the polyvalent sialoside.

The FMO/PCM method as a tool for the residue-based energy analysis is very useful to study the role of key amino acid residue for the influenza viral hemagglutinin bindings to the sialoside receptors in the equilibrium 1:1 binding. In perspectives, the FMO method has collaborated with the other approaches such as the molecular dynamics simulations, the QMMM framework, and the empirical amino acid sequence alignment that can help us to understand the mechanism of the new viral HA binding to sialoside.

ABBREVIATIONS:

HA	=	hemagglutinin
Neu5Acα2-6Gal	=	*N*-acetyl-D-neuraminic acid α2-6 β-D-galactose
Neu5Acα2-3Gal	=	*N*-acetyl-D-neuraminic acid α2-3 β-D-galactose
FMO	=	fragment molecular orbital
BHA	=	bromelain-released hemaggulinin
NMR	=	nuclear magnetic resonance
Neu5Acα2-6Galβ1-4Glc	=	*N*-acetyl-D-neuraminic acid α2-6 D-galactose β1-4 β-D-glucose
Neu5Acα2-3Galβ1-4Glc	=	*N*-acetyl-D-neuraminic acid α2-3 D-galactose β1-4 β-D-glucose
MD	=	molecular dynamics
HF	=	the Hartree-Fock method
MP2	=	the second-order Møller-Plesset perturbation theory
PCM	=	polarizable continuum model

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