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Computational studies of H5N1 hemagglutinin binding with SA-α-2, 3-Gal and SA-α-2, 6-Gal

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Abstract

For influenza H5N1 hemagglutinin, a switch from SA-α-2, 3-Gal to SA-α-2, 6-Gal receptor specificity is a critical step leading to the conversion from avian-to-human to human-to-human infection. Therefore, the understanding of the binding modes of SA-α-2, 3-Gal and SA-α-2, 6-Gal to H5N1 hemagglutinin will be very important for the examination of possible mutations needed for going from an avian to a human flu virus. Based on the available H5N1 hemagglutinin crystal structure, the binding profiles between H5N1 hemagglutinin and two saccharide ligands, SA-α-2, 3-Gal and SA-α-2, 6-Gal, were investigated by *ab initio* quantum mechanics, molecular docking, molecular mechanics, and molecular dynamics simulations. It was found that SA-α-2, 3-Gal has strong multiple hydrophobic and hydrogen bond interactions in its *trans* conformation with H5N1 hemagglutinin, whereas the SA-α-2, 6-Gal only shows weak interactions in a different conformation (*cis* type).

Keywords: H5N1; Avian influenza; Neuraminidase; Hemagglutinin; Molecular docking; *ab initio* calculation; Molecular mechanics; Molecular dynamics

The outbreak of H5N1 avian influenza virus, or commonly called "bird flu," is of a major health concern not only because of its high death rate [1], but also because of its highly contagious nature and its ability to mutate and develop resistance to known therapies [2]. The possibility of a human pandemic of H5N1 flu is not considered a remote possibility [3] if uncontrolled. Therefore, there is a great deal of interest in examining the various factors important for the transformation of a virus that primarily infects chicken to a strain that would pass from human to human.

As one of the two principal antigens found on the influenza viral surface, hemagglutinin (HA) interact with host-cell receptors containing the terminal sialic acid (SA) residue [4]. Such interactions are responsible for viral binding to host cells, enabling cellular entry through endocytosis. Therefore, HA could be an important target for both drug and vaccine development [5]. SAs are usually found in either an α-2, 3 or an α-2, 6 linkage to galactose (Gal), the predominant penultimate sugar of N-linked carbohydrate side chains (Fig. 1) [6]. Human influenza viruses prefer SA-α-2, 6-Gal-linked saccharides, whereas avian influenza viruses prefer those terminating in SA-α-2, 3-Gal [7].

Recently there have been several exciting investigations of H5N1 HA. One is the recognition that H5N1 virus with specificity for SA-α-2, 3-Gal would preferentially attach to the lower respiratory airway in human [8,9]. The other is the resolution of the crystal structure of HA derived from A/Vietnam/1203/2004 (H5N1) virus [10]. It is believed that a switch from α-2, 3 to α-2, 6 receptor specificity is a critical step in the adaptation of avian viruses to a human host, while α-2, 3 specificity alone appears to be one of the reasons that most avian influenza viruses, including current avian H5 strains, are not easily transmitted from human to human after avian-to-human infection [7,11]. Thus, the question that needs to be addressed is how a H5N1 virus

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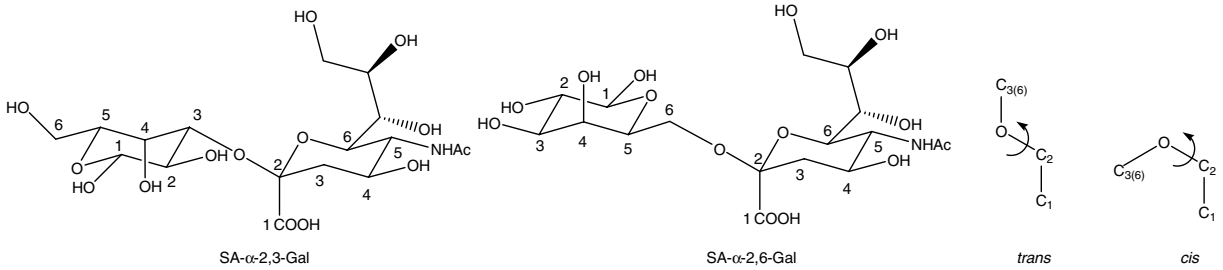

Fig. 1. The chemical structures of SA-α-2, 3-Gal and SA-α-2, 6-Gal.

could adapt its HA for binding with human receptor, SA-α-2, 3-Gal and SA-α-2, 6-Gal.

In this study, we undertook the task of using computational methods to understand the binding of H5N1 HA to SA-α-2, 3-Gal and SA-α-2, 6-Gal. First of all, the ligands were built and optimized by *ab initio* calculation. Subsequently, the ligands were docked into the receptor site of the crystal structure of H5N1 HA. The complexes were then optimized by molecular mechanics and molecular dynamics approaches. Finally, the optimized complexes were analyzed in terms of ligand-HA interactions. The results from this study should allow for a better understanding of the binding mode of H5N1 HA with SA-α-2, 3-Gal and SA-α-2, 6-Gal. Such information should be very useful for understanding mutations that could lead to human infection and for the design of inhibitors that could block the binding of H5N1 virus to host cells.

Materials and methods

Modeling of SA-α-2, 3-Gal and SA-α-2, 6-Gal binding with HA. The ligands, SA-α-2, 3-Gal and SA-α-2, 6-Gal, were built and optimized at Hartree-Fock level with the 6-311 G basis set by Gaussian 03 program [12]. The optimized ligands were then embedded with Gastiger-Hückel partial charge by SYBYL 7.1 package. For HA, the original crystallographic structure was used as a starting point (PDB entry: 2FK0) [10], with the addition of all missing hydrogen atoms and assignment of Kollman all-atom charges by SYBYL. Docking of the ligands into the HA receptor site was then performed by DOCK 5.4 program [13]. The docked complexes were solvated by using the TIP3P water model, subjected to 500-steps of molecular mechanics minimization and molecular dynamics simulations at 300 K for 1.5 ns using the SANDER module in AMBER 8 program [14]. The resultant structures were then analyzed using HBPLUS 3.06 [15] and Ligplot 4.22 [16] program to identify specific contacts between ligands and HA.

Hardware and software. SYBYL 7.1 was used for molecular modeling on a SGI workstation. The *ab initio* optimization (Gaussian 03), molecular mechanics calculations, and molecular dynamics simulations (AMBER 8) were performed on a Linux-based 40-node cluster. The docking calculation (DOCK 5.4) and binding analysis (HBPLUS 3.06 and Ligplot 4.22) were carried out on a Linux workstation. The visualization of complexes was employed by Pymol 0.99 program [17] on a Windows XP workstation.

Results and discussion

SA-α-2, 3-Gal-H5N1 HA complex analysis

Since H5N1 HA has an intrinsic preference for SA-α-2, 3-Gal [7,11], we first studied the binding of H5N1 HA with SA-α-2, 3-Gal. In doing so, the structure of SA-α-2, 3-Gal was first derived from *ab initio* calculations. This optimized SA-α-2, 3-Gal structure was docked into the binding site of H5N1 HA and then minimized using both molecular dynamics and molecular mechanics in the TIP3P soaked model. The schematic analysis of SA-α-2, 3-Gal-H5N1 HA complex shows the residues involved in receptor site as seen in Fig. 2. The docking conformation of SA-α-2, 3-Gal around the receptor binding domain of H5N1 HA is depicted in Fig. 3. In the optimized structure, SA-α-2, 3-Gal adopted a U-shape with the two monosaccharides in a *trans* orientation (for *cis* and *trans* definition, see Fig. 1). On the whole SA-α-2, 3-Gal has possible strong hydrophobic interaction with seven amino acid residues, Ser 136, Trp 153, Ile 155, His 183, Glu 190, Leu 194, and Gln 226, as judged by the HBPLUS program [15]. Moreover, analysis with the HBPLUS program suggests that SA-α-2, 3-Gal can form 11 strong hydrogen bonds with Tyr 98, Val 135, Ser 136, Ser 137, His 183, Glu 190, and Gln 226. The *trans* conformation of SA-α-2, 3-Gal directs the Gal ring somewhat away from the receptor binding domain surface. As a result, there is only partial interaction of the Gal moiety with the receptor. However, there is a strong hydrogen bond between the axial 4-hydroxyl group of Gal and the important Gln 226 residue (carbonyl oxygen), which is also involved in hydrogen bond interactions with the 1-carboxylate and 2-glycerol hydroxyl group of SA. Our results are consistent with what has been proposed based on experiments, i.e., Gln 226 is a very critical residue involved in H5N1 HA receptor binding [18]. Overall, the results indicate that SA-α-2, 3-Gal have strong interactions and thus binding with H5N1 HA, which are consistent with experimental results [8,9].

SA-α-2, 6-Gal-H5N1 HA complex analysis

Similar to the case of SA-α-2, 3-Gal-H5N1 studies, the initial conformation of SA-α-2, 6-Gal was derived from *ab initio* calculations. This structure was then docked into the receptor binding site of H5N1 HA. The minimized structure of the complex showed SA-α-2, 3-Gal in a similar general orientation as compared with SA-α-2, 3-Gal. Furthermore, compared with the *trans* conformation of SA-α-2, 3-Gal, one most prominent feature is that SA-α-2, 6-Gal adopts a *cis* conformation. The hydrophobic