Molecular docking of sialic acid and its sialic acid-gadolinium (III) poliaminocarboxylate complex conjugate, respectively, toward hemagglutinin of H5N1 viruses of Vietnam

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Abstract

Avian Influenza is one of the infection disease that still become a complex and serious health problem. This disease particularly is caused by H5N1 virus which the spikes of virus contain 2 main proteins that’s call hemagglutinin and neuraminidase. Hemagglutinin is antigenic glycoprotein that can be found on the surface of influenza virus which role bind virus to host cell through sialic acid molecule. The goal of this research is to calculate the binding energy value GdDTPA sialic acid conjugate molecule and determine amino acid residues which give contribution happen hydrogen bonding and hydrophobic interaction in docking process sialic acid and GdDTPA sialic acid conjugate molecule with hemagglutinin as preliminary study for design MRI contrast agent compound to diagnose avian influenza patient suspect by using MRI contrast agent. Three dimension structure of GdDTPA sialic acid conjugate molecule was modelled with MM2 method through Chem 3D software. Then docking simulation was carried out between sialic acid and conjugate molecule with hemagglutinin active site to determine binding energy value and the amino acid residues on binding site that can be found hydrogen bonding and hydrophobic interaction by using Chem 3D and Arguslab software. From this research, binding energy predicted value of conjugate molecule is -4.26845 kcal/mole with 2 hydrogen bonding interactions on Gln226 and Ser227 and 1 hydrophobic interaction on Gln226.

Keywords: Hemagglutinin, H5N1 virus, sialic acid, GdDTPA, docking

Introduction

Over the past century, emergence of epidemic influenza has been serious threat to human health. The origin of human influenza viruses is thought to be avian influenza virus because all the subtypes are found in avian host. Newly adapted avian influenza virus to human host or reassortant virus could be pandemic because we have no immunity for it, as shown in our history such as 1918 (H1N1), 1957 (H2N2) and 1968 (H3N2) pandemics. Recently, the first H5 avian influenza virus infected patient was reported and emergence of new pandemic influenza is alerted (WHO, 2006).

Influenza viruses are pleomorphic, enveloped RNA viruses belonging to the family of Orthomyxoviridae. Protruding from the lipid envelope are two distinct glycoproteins, the hemagglutinin (HA) and neuraminidase (NA). HA attaches to cell surface sialic acid receptors, thereby facilitating entry of the virus into host cells. Since it is the most important antigenic determinant to which neutralizing antibodies are directed, HA represents a crucial component of current vaccines. NA is the second major antigenic determinant for neutralizing antibodies. By catalyzing the cleavage of glycosidic linkages to sialic acid on host cell and virion surfaces, this glycoprotein prevents aggregation of virions thus facilitating the release of progeny virus from infected cells. Inhibition of this important function represents the most effective antiviral treatment strategy to date (de Jong & Tinhien, 2006).

Hemagglutinin (HA), the principal antigen on the viral surface, is the primary target for neutralizing antibodies and is responsible for viral binding to host receptors, enabling entry into the host cell through endocytosis and subsequent membrane fusion. As such, the HA is an important target for both drug and vaccine development. Although 16 avian and mammalian serotypes of HA are known, only three (H1, H2, and H3) have become adapted to the human population. HA is a homotrimer; each monomer is synthesized as a single polypeptide (HA0) that is cleaved by host proteases into two subunits (HA1 and HA2). HA binds to receptors containing glycans with terminal sialic acids, where their precise linkage determines species preference. A switch in receptor specificity from sialic acids connected to galactose in α2-3 linkages (avian) to α2-6 linkages (human) is a major obstacle for influenza A viruses to cross the species barrier and to adapt to a new host (Shiya et al., 2005; Suzuki et al., 2000). On H3 and H1 HA frameworks, as few as two amino acid mutations can
switch human and avian receptor specificity (Stevens et al., 2006).

Gadolinium ion (Gd\(^{3+}\)) is very suitable for MRI contrast agent compound because of paramagnetic properties and stabilization of its chelat. Gadolinium complex molecules are paramagnetic complex that can be used widely as MRI contrast agent compound. Some factors which cause Gd complex suitable for this application are Gd\(^{3+}\) ion have highest paramagnetic properties because of seven unpair electron in 4\(^{f}\) valence shell, have longest electronic relaxation time, and can form stable chelate that can coordinate with two water compound (inner-sphere coordination) (Aime et al., 2002). Some example of Gd\(^{3+}\) complex compounds that have been accepted in clinical study are Gd-DTPA, Gd-BOPTA, Gd-DOTA (Jacques & Desreux, 2002).

Recently, avian influenza viruses have been infected human as host cell and cause many suspected patient bird flu died in large number. For diagnose the suspected patient bird flu, some hospital are still using laboratory procedure usually performed by immunochromatographic or immunofluorescent detection of influenza virus antigens, or reverse transcriptase polymerase chain reaction (RT-PCR) detection of viral nucleic acids in respiratory specimen (Hien et al., 2004). This method is takes a lot of time (4 to 5 days) to have complete diagnose. So, we study the stabilization and binding affinity of GdDTPA sialic acid conjugate compound through computational chemistry approach for novel MRI contrast agent compound to diagnose suspected human bird flu by using MRI contrast agent.

**Materials and Methods**

**Modeling of sialic acid and sialic acid – GdDTPA conjugate molecule.**

The native sialic acid molecule was modeled by conjugate the 3D structure of CHx-A''-Gd-DTPA using Chem 3D 8 software. Structure of sialic acid and conjugate molecule GdDTPA – sialic acid were done minimisation energy job using molecular mechanic (MM2) method. Then, conjugate molecule GdDTPA – sialic acid was modified with replaced the electron donor atom (N atom) of conjugate molecule to dummy atom (Du atom). Both of structure minimisation result were calculate the steric energy summary by using compute properties job and done the overlay structure between those of structures. The parameter result from minimisation i.e. length bond and angel bond were calculated the root mean square (RMS) value to find out the deviation value between both structures. Then, validation docking method was carried out by docking the native sialic acid into receptor binding site (RBS) of HA by using ArgusLab 4 software. Validation method was done to compare the variation of grid resolution and docking method in ArgusLab 4. Docking of ligand sialic acid and conjugate molecule into the HA (PDB:2FK0) receptor binding site was performed with grid resoution and docking method result from validation docking method. Binding energy (ΔG) result was compared and the best conformation result of both ligands were analyzed the hydrogen bonding and hydrophobic interaction to identify specific contact between ligands and HA.

**Results and Discussion**

**Modeling of sialic acid – GdDTPA conjugate molecule**

Complex struture of CHx-A''-Gd-DTPA compound was drawed with chem draw software to create the 2 dimension structure of GdDTPA. The 2 dimension structure of GdDTPA compound was exported to 3 dimension structure by using chem 3D software. The result compound of GdDTPA structure complex can be seen in Fig.1.

![Figure 1](image) The 3D strucure of GdDTPA complex compound.

The native structure of sialic acid was separated from the protein HA co – crystallize structure. Then sialic acid structure was conjugated with GdDTPA structure through connecting the Nitrogen atom of imine tail GdDTPA structure to Carbon atom 1 (C-1) of sialic acid structure which have been removed the hydroxyl tail (-OH) bind to C-1 atom before. The result of conjugate structure sialic acid – GdDTPA is showed in Fig.2.
Then, conjugate structure and sialic acid native structure were done minimisation energy job using molecular mechanic MM2 method to make the stable conformation of both structures based on intramolecular interaction among the atoms composition of both molecules. The result of minimisation energy was stable conformation structures of sialic acid and conjugate molecule as we seen in Fig.3.

Conjugate molecule GdDTPA – sialic acid was modified with replacing the electron donor atom (Nitrogen atom) to dummy atom and remove the GdDTPA structure complex. This procedure was done to know how much the deviation of sialic acid conformation of conjugate molecule to native sialic acid that have been done minimisation energy before. The modified conjugate molecule and sialic acid structure were done overlay to know the different conformation of sialic acid in conjugate molecule. From the overlay structure, we see that there is a little different conformation among those structures which is showed in Fig.4. The atoms Carbon no. 11, 12, 13, 14, 15, 16, 17, 18, and 20 show that overlapped each other and also for Oxygen atoms no. 2, 3, 4, 5 and Nitrogen atom no. 10. Overall, the conformation of sialic acid structure from conjugate molecule is close to sialic acid native structure. This result is agree with calculation result of RMS (Root Mean Square) length bond and angle bond. The RMS of length bond is 0.004685 and angle bond is 1.1.9265. This calculation result show that the RMS value of length bond and angle bond are small. It’s mean that both sialic acid structures have close conformation structure. So, there is a little bit deviation between both sialic acid structures.

After that, both sialic acid structures were calculated the steric energy summary to know the stabilization of both compound. The calculations result of steric energy summary are shown in Tab.1.

**Tabel 1 The sum of steric energy and its composition energy**

<table>
<thead>
<tr>
<th>Composition of steric energy</th>
<th>Conjugate sialic acid (kcal/mole)</th>
<th>Native sialic acid (kcal/mole)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stretch</td>
<td>5.7070</td>
<td>5.6029</td>
</tr>
<tr>
<td>Bend</td>
<td>24.0232</td>
<td>28.9564</td>
</tr>
<tr>
<td>Stretch-Bend</td>
<td>0.9994</td>
<td>1.2446</td>
</tr>
<tr>
<td>Torsion</td>
<td>11.6823</td>
<td>10.7191</td>
</tr>
<tr>
<td>Non-1,4 VDW</td>
<td>-8.7975</td>
<td>-10.7716</td>
</tr>
<tr>
<td>1,4 VDW</td>
<td>15.6697</td>
<td>13.6970</td>
</tr>
<tr>
<td>Dipole/Dipole</td>
<td>-3.8840</td>
<td>-6.2429</td>
</tr>
<tr>
<td>Total</td>
<td>45.4001</td>
<td>43.2056</td>
</tr>
</tbody>
</table>

The result indicate that total steric energy of conjugate molecule is less stable than native sialic acid, because of calculation show that the steric energy total of sialic acid conjugate molecule (45.4001 kcal/mole) is bigger than sialic acid native structure (43.2056 kcal/mole). Value of steric energy composition for bend energy, non-1,4 VDW energy, and dipole/dipole energy show big differences. This result impact on the imperfect result of the overlay structure between both sialic acid molecules.
Validation docking method in ArgusLab software.

Validation docking in ArgusLab was carried out for validate the docking method and the variation of grid resolution value in docking simulation with ArgusLab. This procedure was used to know how does the effect of the variation docking method and grid resolution value in the result of docking simulation with ArgusLab. We compare the docking method in ArgusLab between ArgusDock and GADock method and also the variation of grid resolution (0.1; 0.15; 0.2; 0.25; 0.3; 0.35; 0.4 Å).

Both docking methods (ArgusDock and GADock) have different approximation. For ArgusDock, the docking method use structure and ligand approximation which is docking mechanism directed to limited position only. Meanwhile, GADock use approximation which ligand is docking directed to all possible position in receptor (protein) (9). This validation procedure base on the different value of RMSD (Root Mean Square Deviation) between native sialic acid ligand and copy sialic acid ligand which dock into the receptor binding site of hemagglutinin. From this validation, we can get the best docking method and value of grid resolution based on the smallest value of RMSD. In Tab.2, we can see the result of validation docking method.

<table>
<thead>
<tr>
<th>Grid resolution (Å)</th>
<th>RMSD value of docking method</th>
<th>GADock</th>
<th>ArgusDock</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1</td>
<td>0.963723</td>
<td>4.146226</td>
<td></td>
</tr>
<tr>
<td>0.15</td>
<td>1.270897</td>
<td>4.247986</td>
<td></td>
</tr>
<tr>
<td>0.2</td>
<td>0.614733</td>
<td>4.397956</td>
<td></td>
</tr>
<tr>
<td>0.25</td>
<td>0.867363</td>
<td>4.246813</td>
<td></td>
</tr>
<tr>
<td>0.3</td>
<td>1.046308</td>
<td>4.403548</td>
<td></td>
</tr>
<tr>
<td>0.35</td>
<td>0.711158</td>
<td>4.391381</td>
<td></td>
</tr>
<tr>
<td>0.4</td>
<td>1.024216</td>
<td>4.458099</td>
<td></td>
</tr>
</tbody>
</table>

The docking validation method is valid if the RMSD value is not more than 2.0000. From this result, we can conclude that the smallest RMSD value was 0.614733. GADock docking method has smaller RMSD value than ArgusDock. So, GADock is much better used in docking procedure than ArgusDock. The variation value of grid resolution show that 0.2 Å give the smallest RMSD value in GADock docking method. In this case, we use GADock as docking method and 0.2 Å as the value of grid resolution for running docking simulation sialic acid native structure and sialic acid conjugate molecule into the receptor binding site of protein hemagglutinin.

Docking simulation and interaction analysis of sialic acid native structure with protein hemagglutinin

Docking simulation between sialic acid native structure and protein hemagglutinin were carried out with ArgusLab software. The parameter of calculation was using parameter data from validation docking result. In this case, we use protein hemagglutinin Vietnam (PDB code:2FK0) which is the host sel come from human bird flu case. The result of docking simulation (in Fig.5a) show that the best pose of native sialic acid structure with predicted binding energy value (ΔG) -3.5443 kcal/mole. This docking simulation create 13 poses of native sialic acid structure and the best pose indicate that sialic acid give the best conformation which fit into receptor binding site of protein hemagglutinin. We also analyse the interaction between ligand native sialic acid and receptor binding site of hemagglutinin protein.

From the Fig.5b, we see that there are 4 hydrogen bonding and 6 hydrophobic interactions which can be formed between sialic acid and hemagglutinin. Hydrogen bonding interactions (red line) are involved 2 amino acid residues i.e. Glu190 and His 193. Meanwhile, hydrophobic interctions (blue line) are involved 3 amino acid residues, that is Leu 194, Gly 228, and Ser 227. Many hydrophobic interactions with protein hemagglutinin this indicate the sialic acid native structure can’t get into the active site of protein hemagglutinin. Hydrogen bonding that is formed between sialic acid and hemagglutinin have a weak interaction and long distance. Overall, from the energy and geometry analysis, the interaction between sialic acid native structure and protein hemagglutinin show weak interactions.

Docking simulation and interaction analysis of sialic acid conjugate structure with protein hemagglutinin

For sialic acid conjugate structure, docking simulation used the same parameter data from docking validation parameter that have been done before. The different procedure from docking sialic acid native structure is treatment of ligand. We subjected ligand as rigid ligand For sialic acid conjugate structure. Beside that, sialic acid native structure is treated as flexible ligand. This was done in order to sialic acid conjugate molecule can’t change its conformation. So, the docking simulation was running with rigid – rigid docking method. We can see in Fig.6a, predicted binding energy value (ΔG) from docking simulation is about -4.26845 kcal/mole. This value was less than from docking simulation of sialic acid native structure. From energy analysis, it show that sialic acid conjugate molecule could bind tightly in hemagglutinin receptor binding site and also could shift sialic acid native structure.
Figure 5 Result of docking simulation between sialic acid native structure and protein hemagglutinin. (a) The best pose of sialic acid with binding energy value, (b) interaction analysis show red line is hydrogen bonding and blue line is hydrophobic interaction.

The interaction analysis (Fig. 6b) also show that sialic acid conjugate molecule can form 2 hydrogen bonding and 1 hydrophobic interaction with amino acid residues in hemagglutinin receptor binding site. Hydrogen bonding interactions are involved 2 amino acid residues Gln 226 and Ser 227 which is Gln 226 have strong interaction (2.473235 Å) with sialic acid conjugate molecule. Experiment result from Taiwan researcher show Gln 226 is a critical amino acid residue that can make stronger interaction with SA-α-2, 3-Gal than SA-α-2, 6-Gal (Li & Wang, 2006). Hydrophobic interaction only can form 1 interaction with Gln 226 amino acid. This interaction indicate sialic acid conjugate molecule can get into hemagglutinin active site. Because from hydrophobic interaction only show fewer interaction than sialic acid native structure. This interaction analysis can be concluded that sialic acid conjugate molecule bind tightly than sialic acid native structure.

Figure 6 Result of docking simulation between sialic acid conjugatemolecule and protein hemagglutinin. (a) The best pose of sialic acid with binding energy value, (b) interaction analysis show red line is hydrogen bonding and blue line is hydrophobic interaction.
Conclusions

In summary, we have determined how sialic acid native structure and sialic acid conjugate molecule bind with hemagglutinin H5N1 virus using molecular mechanic (MM2) calculation and molecular docking simulation. Given the results presented in this report, it indicates that the sialic acid conjugate molecule has strong hydrogen bond interactions whereas sialic acid native structure only shows weak interactions. Most of the difference arise from interaction with residue Gln 226 amino acid that have strong hydrogen bonding with sialic acid conjugate molecule. From hydrophobic interactions, sialic acid conjugate molecule show only a few interaction with amino acid residues. This show that sialic acid conjugate molecule can be get into the active site of hemagglutinin protein. So, we can conclude sialic acid – GdDTPA conjugate molecule can shift the native structure of sialic acid and also this conjugate molecule can be suggested as MRI contrast agent compound for diagnose suspected human bird flu disease.

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References


