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Influenza viral hemagglutinin complicated shape is advantageous to its binding affinity for sialosaccharide receptor

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Abstract

Do the complexity and the bulkiness of a protein affect the affinity between protein and ligand? We attempted to investigate this problem by using ab initio fragment molecular orbital (FMO) method to calculate the binding energy between human influenza viral hemagglutinin (HA) and human oligo-saccharide receptor. We compared the binding energies of 4 different sizes of human A virus HA H3 subtype complexed with human receptor Neu5Ac(α2-6)Gal as a model. The full shape receptor binding domain complexed with Neu5Ac(α2-6)Gal had the highest binding energy 170.3 kcal/mol at the FMO-HF/STO-3G level, which was 52.3 kcal/mol higher than that of the smallest domain-receptor complex. These data provide the consideration of the backyard bulkiness beyond the binding site of protein to the protein-ligand stability.

Keywords: Influenza; Hemagglutinin; Neu5Ac(α2-6)Gal; Ab initio FMO; Binding energy

Proteins are large and complicated molecules which closely concern with biochemical events in vivo. Biochemists have always been wondered why proteins take such intricate shapes and how much these shapes have the advantage to the biochemical reactions. Very recently, Boehr et al. have demonstrated that dynamic fluctuations in dihydrofolate reductase conformation govern its Catalytic turnover rate [1,2].

With the advent of computer technology, we have been able to evaluate the biochemical interactions in silico such as protein-protein or protein-ligand interactions. For the assessment of protein-ligand interactions, we usually select the limited atoms on ligand binding pockets for the computations. However, the adequacy of clipping the protein parts has not been theoretically understood. Here we attempted to evaluate the protein bulkiness by using ab initio fragment molecular orbital (FMO) calculations for the binding energy between the influenza virus and its oligosaccharide receptor.

Influenza virus hemagglutinin (HA) is one of the lectin protein, which binds to sialo-glycolipids and sialo-glyco-

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proteins on target cell surface as the first step of virus infection [3,4]. HA strictly recognizes the difference in sialic acid-galactose linkage and the sialic acid species [5–10]. Skehel and Wiley et al. have already reported the X-ray crystallographic structures of various influenza HA complexed with Neu5Ac(α2-3 or α2-6)Gal receptors [11,12]. HA forms a trimer and each monomer consists of receptor binding domain and membrane fusion domain as indicated in Fig. 1 [13,14]. Why does the receptor binding domain take such a complicated shape? How much advantage does the shape has to its binding affinity? In this paper, we have quantitatively demonstrated the domain size dependency of binding energy between human influenza A virus hemagglutinin H3 subtype and human receptor Neu5Ac(α2-6)Gal by ab initio FMO methods.

Fig. 1. Structure of human influenza A virus H3 monomer complexed with human receptor Neu5Ac(α2-6)Gal. Receptor binding domain: colorful ribbon, membrane fusion domain: yellow ribbon, Neu5Ac(α2-6)Gal receptor: CPK model.

As shown in Fig. 2, we treated three different sizes of the receptor binding domains **A**–**C**, full size domain **D**, and the modified shape **B**–**A** in the complex of human A virus H3 with Neu5Ac(α2-6)Gal to estimate its influence on the binding energy. These complexes were prepared from the energy minimized structure of human virus H3 trimer complexed with three Neu5Ac(α2-6)Gal analogs (1509 amino acid residues, 24,519 atoms) [13,14] by molecular mechanics calculations with CFF force field [15] implemented in Discovery Studio 1.5.1 program (Accelrys Inc.). We computed the single point energies of the complexes (E_{complex}), corresponding H3 receptor binding domains (E_{H3}), Neu5Ac(α2-6)Gal receptors (E_{receptor}) at the FMO-HF/STO-3G level to calculate binding energies (ΔE) by the following equation: $\Delta E = (E_{\text{H3}} + E_{\text{receptor}}) - E_{\text{complex}}$. Ab initio FMO calculations were carried out using ABINIT-MP program [16] and the target molecules were treated as similar to our previous work [17]. H3 in the complexes was divided into single amino acid residues as fragments and Neu5Ac(α2-6)Gal receptor was also treated as a single fragment. The receptors and H3s charged to −1 and +1, respectively.

The binding energies at the FMO-HF/STO-3G level in the complexes are summarized in Table 1. The full shape of H3 receptor binding domain clearly benefits to the binding between H3 and Neu5Ac(α2-6)Gal receptor. Full size complex **D** has the highest binding energy 170.3 kcal/mol, and which is 52.3 kcal/mol larger than that of complex **A** containing the smallest receptor binding domain. Binding energy in the complex **B** is also 40.0 kcal/mol larger than that of complex **A**. Amino acid residues far away from the receptor binding site in the complexes **B**,**C**, and **D** are not interacting directly with Neu5Ac(α2-6)Gal, but they can influence the binding energy via the smallest receptor binding site by forming various interactions like hydrogen bond network, lipophilic interaction, π–π stacking and so on. In addition, there was no binding energy in the modified complex **B**–**A** which is supporting our suggestion. The binding energy in complex **C** is 13.4 kcal/mol smaller than that of complex **B**. Full size complex **D** has 12.3 kcal/mol binding advantage toward complex **B**, and the advantage is 27.7 kcal/mol smaller than that of **B** to **A**. These results reveal that amino acid residues near the receptor binding site strongly affect the binding energy and optimum protein size is necessary for stabilization of protein-ligand complex.

In this paper, we have demonstrated that the complicated shape of human influenza virus hemagglutinin H3 manifests to its binding affinity to human receptor Neu5Ac(α2-6)Gal at the FMO-HF/STO-3G level. These data show that the structure beyond the binding site significantly influences the protein-ligand affinity. It may also help in the understanding the relevance of the shape,

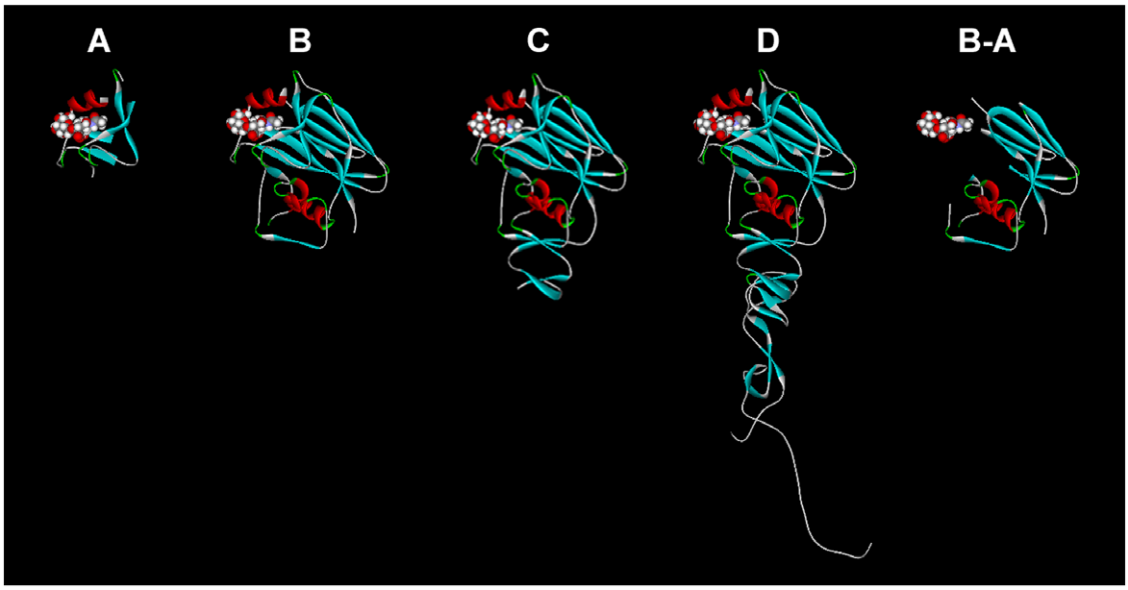

Fig. 2. The complexes of human influenza A virus H3 receptor binding domain with human receptor Neu5Ac(α2-6)Gal for the binding energy calculations. **A**: The complex has the smallest receptor binding domain (N96-P99, G129-Y161, G181-V196, and N216-I232, 70 residues, 1113 atoms) whose size is treated similarly as that of our previous study [17]. **B**: Binding domain in the complex consists of I62-G263 (202 residues, 3183 atoms). **C**: The domain involves G49-T283 (235 residues, 3676 atoms). **D**: The complex has the full size of receptor binding domain (Q1-T328, 5068 atoms). **B**–**A**: The modified complex is prepared by cutting out the binding domain in complex **A** from complex **B** (132 residues, 2155 atoms). The complex has no amino acid residues which directly interact with Neu5Ac(α2-6)Gal.

Table 1
The binding energies ΔE between human H3 and human receptor Neu5Ac(α2-6)Gal in the complexes at the FMO-HF/STO-3G level

	H3-Neu5Ac(α2-6)Gal complex				
	A	**B**	**C**	**D**	**B–A**
Binding energy, ΔE (kcal/mol)	118.0	158.0	144.6	170.3	−1.3

complexity and the bulkiness of protein to the protein-ligand complex stability.

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