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Hydration of ligands of influenza virus neuraminidase studied by the fragment molecular orbital method

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ABSTRACT

The fragment molecular orbital (FMO) method was applied to quantum chemical calculations of neuramic acid, the natural substrate of the influenza virus neuraminidase, and two of its competitive inhibitors, Oseltamivir (Tamiful®) and Zanamivir (Relenza®), to investigate their hydrated structures and energetics. Each of the three ligands was immersed in an explicit water solvent, geometry-optimized by classical MM and QM/MM methods, and subjected to FMO calculations with 2-, 3-, and 4-body corrections under several fragmentation options. The important findings were that QM/MM optimization was preferable to obtain reliable hydrated structures of the ligands, that the 3-body correction was important for quantitative evaluation of the solvation energy, and that the dehydration effect was most remarkable near the hydrophobic sections of the ligands. In addition, the hydration energy calculated by the explicit solvent was compared with the hydration free energy calculated by the implicit solvent via the Poisson-Boltzmann equation, and the two showed a fairly good correlation. These findings will serve as useful information for rapid drug design.

1. Introduction

In this article, we present quantum chemical studies on three ligands of the influenza virus neuraminidase (NA), namely, N-acetylneuraminic acid (Neu5Ac), active form of Oseltamivir, and Zanamivir (Fig. 1). We aim to understand their electronic structures after hydration because dehydration of the ligand occurs at the beginning of the ligand-protein complex formation. Hence we seek to design a potent medicine by minutely modifying the hydration properties of the ligands to promote their association with NA.

NA resides on the surface of the influenza virus and is an important target enzyme of anti-influenza medicines. NA recognizes and cleaves the terminal sialic acid residue of the receptor protein to propagate progeny virions from infected cells [1]. This important role of NA in the early stage of virus infection has led to the idea of designing novel NA inhibitors as anti-virus medicines. Quite a few molecules are known to bind to NA, but have chosen Neu5Ac, Oseltamivir, and Zanamivir because of their vast experimental information, especially their crystal structures complexed with NA.

Neu5Ac, the natural substrate of NA, has been selected as a template molecule in drug design. Neu5Ac is the major sialic acid found in mammalian cells, usually in the form of glycoprotein or gangliosides. The determination of the crystal structures of the complex of NA and Neu5Ac [2,3] has provided detailed information about the interaction of Neu5Ac with surrounding amino acid residues [4,5] (Fig. 2) and has promoted computer-aided design of NA inhibitors including Oseltamivir (Tamiful®) [6] and Zanamivir (Relenza®) [7]. Oseltamivir, Zanamivir, and similar anti-NA inhibitors have proven effective as anti-influenza medicines [1,5,8,9]. Along with the emergence of drug-resistant viruses, such novel anti-influenza medicines are increasingly in demand.

Collins et al. [10] reported the crystal structures of mutant NAs of Oseltamivir-resistant viruses. The structures suggest that interactions between amino acid residues of NA and functional groups

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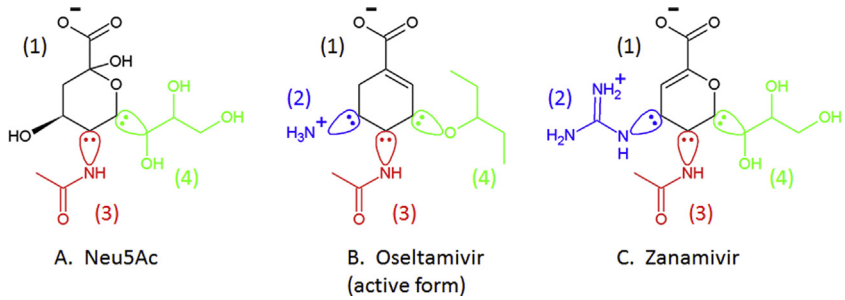

Fig. 1. The three ligand molecules that bind to NA. The fragmentation patterns for FMO are color-coded. Each ligand is divided into four fragments (1)-(4). Neu5Ac lacks fragment (2) because its possible second fragment (OH) is so small that it is merged to fragment (1).

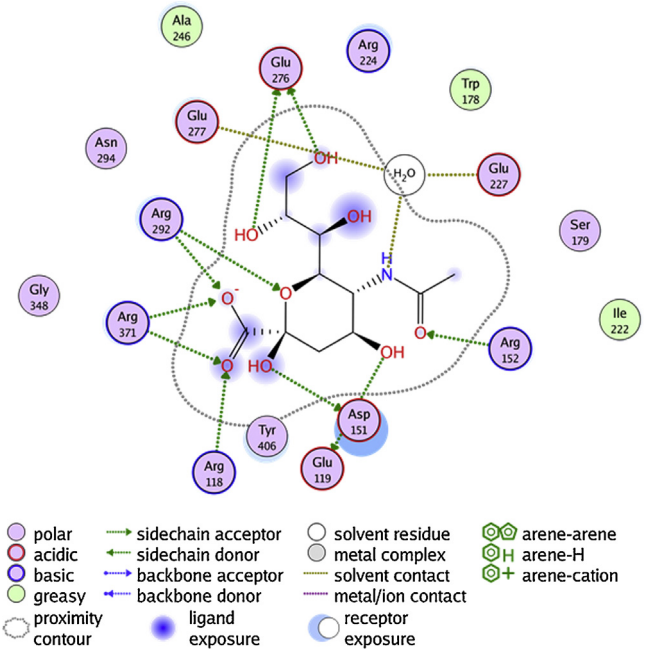

Fig. 2. Interaction of Neu5Ac with surrounding water molecules and amino acid residues of NA. A schematic picture based on the crystal structure of the Neu5AC:NA complex (PDB:2BAT) [4] drawn with MOE [31].

of the ligands play a significant role in the ligand recognition of the protein. This implication, in turn, has led us to focus on the important physical properties of individual functional groups in designing novel medicines.

A number of computational methods are widely used in the field of drug design [11]; here we focus on the *ab initio* Fragment Molecular Orbital (FMO) method [12–14]. FMO is a fragment-based quantum chemical method developed for electronic state calculations of large molecular systems. FMO retains high precision by incorporating many-body effects, is easy to parallelize, and above all is suitable for highlighting the roles of different functional groups of a drug by providing Inter-Fragment Interaction Energy (IFIE) analysis [15–18] to reveal detailed information on interactions among different functional groups of a molecule. See Section 2.3 for further details of the method.

FMO has already been applied to studies related to drug design to combat the influenza viruses. For example, FMO was applied to specific binding of the influenza virus hemagglutinin to sialosaccharide receptors to reveal important conformational changes upon mutation [19–21]. In another series of studies [22,23], the interactions between NA and sialic acid analogues were examined in order to identify their origins. We ourselves are investigating the interaction between H1 subtype of NA and its substrate and inhibitors (Fukuzawa et al., in preparation), hereafter referred to simply as ligands (Fig. 1). This study has made us realize the importance of the solvent effect in realistic and quantitative evaluations of NA–ligand interactions, because ligand binding to protein entails dehydration of the ligand as the initial step.

In this paper, instead of the interactions with the surrounding amino acid residues (Fig. 2), we focus on the interactions of the ligands with surrounding water molecules. Note that FMO has been applied to proteins, DNAs, Na^+ ions, and small organic molecules hydrated in both explicit [24–28] and implicit solvent models [29,30]. Based on these studies, we analyzed the hydration of Neu5Ac, Oseltamivir, and Zanamivir with a view to gaining information applicable to rational drug design. We investigated the protocols for preparation of the hydrated structures, the level of FMO calculations, the roles of functional groups of the ligands in hydration, and the effect of the implicit solvent model. The results gave us useful suggestions for development of anti-Influenza drugs.

2. Methods

We performed the series of calculations as follows. We started with the hydrated structures of the ligands by MM-MD simulation (subsection 2.1) because we had found that MM-optimization of water solvent is effective in preparation of molecular structures for FMO [28]. Next, we chose an MM-MD snapshot from each of the MM-MD trajectories of the hydrated ligands and further optimized it with the Quantum Mechanical/Molecular Mechanical (QM/MM) method regarding the ligand as the QM region to make the ligand and its neighbor more appropriate for FMO (subsection 2.2). The optimized structures were finally subjected to FMO calculations (subsection 2.3). The QM/MM structures of the ligands were then dehydrated and were subjected to the Poisson-Boltzmann (PB) calculation to examine the implicit solvent model (subsection 2.4).

2.1. Preparation of the hydrated structures of the ligands by MM methods

The hydrated structures of Neu5Ac, Oseltamivir, and Zanamivir were prepared as follows using the Molecular Operating Environment program (MOE) [31] as a platform for classical Molecular Mechanics (MM)-based modeling. The initial 3D structures of the ligands were excised from their crystal structures complexed with NA. We used these complexed structures of the ligands because we are planning to compare the FMO results of the ligands in the isolated state (this study) and in the complexed state (Fukuzawa et al., in preparation) in similar conformations. The original PDB files were 2BAT for Neu5Ac [4], 3CL0 for Oseltamivir [10], and 3CKZ for Zanamivir [10]. Missing hydrogen atoms were added to the excised ligands and were energy-minimized with MM force-field parameters automatically generated by the MOE program (SI-Table 1). Then each ligand was solvated by a sphere of TIP3P water whose