Protonation of an α-Hydroxytropolone HIV RNase H Inhibitor through QM/MM Methods

Rongmei (Judy) Wei

Advisor: Emilio Gallicchio*

PhD Chemistry Program, Graduate Center, City university of New York

Biochemistry Department, Brooklyn College

1. Introduction

The most challenging task in pharmaceutical applications is to discover specific drug derivatives which are most likely to bind strongly to a target receptor.[1] Scientists in related fields have been intensively working on the binding preferences trying to divulge the binding mechanism to practical applications. Here, we are interested in studying an inhibitor of the human immunodeficiency virus (HIV)-1 RNase H, which could be used as a potential drug to inhibit the processing of the DNA/RNA hybrid of HIV-1 by the RNase H enzyme. The RNase H domain of the HIV reverse transcriptase (RT) performs the degradation of DNA/RNA hybrid in the reverse transcriptase (RT) process. Inhibiting the binding function of HIV RT prevents HIV from viral replication.[2] So far, the understanding regarding how the binding occurs is still unclear and not much study progress has been made. Particularly, the HIV RNase H active site is rather shallow and difficult to target by small-molecule inhibitors.[3] In addition, the lack of specificity could cause toxicity with unwanted binding between HIV inhibitors with other similar HIV RNase H enzymes.[1] Given the reasons above, the available data for HIV RNase H inhibitor binding remains inconclusive and limited for current research applications.

Modern structure-based computer-aided drug design methods have become standard practice in drug-discovery fields across both academia and industry. These help lead to a different perspective in terms of guiding chemical synthesis.[4] The fundamental idea in computational simulation methods is to apply available crystallographic models to implement in the molecular mechanic system and calculate the binding strength and conformation variations, therefore, to predict the possible binding mechanisms.

In this project, we intend to study the bound structure of β-thujaplicinol, a member of a class of organic molecules called α-hydroxytropolones, which have been shown to inhibit HIV RNase H. We aim to provide a further insight into the physicochemical properties of RNase H active site to guide drug design studies.

In α-hydroxytropolone HIV RNase H molecule, metal Mg serves as cofactor to active the enzyme, but the protonation state of the oxygen substituents is still not known. Here we proposed

four different states with doubly deprotonated state, shown in Figure 1, a), as well as three singly hydrogen protonated state in the three oxygen positions of tropolone, marked as left, Figure 1, b), middle, Figure 1, c), and right, Figure 1, d), respectively. We therefore perform QM/MM geometry optimizations to find out which protonation state assignment is most consistent with the known crystal structure.

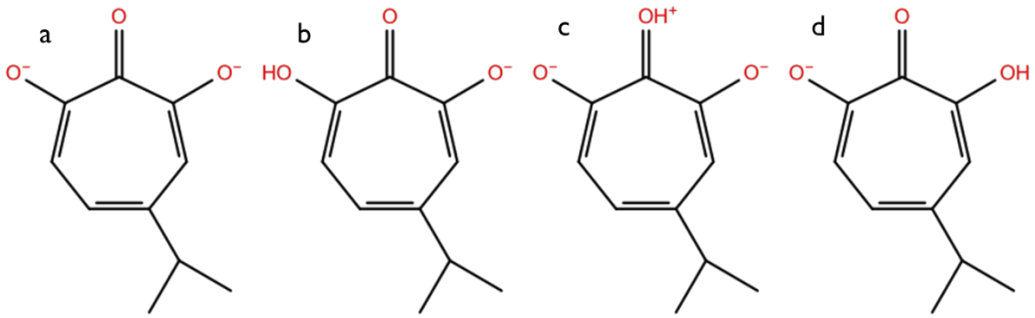

Figure 1. β-thujaplicinol ligand structures in four different states: a) doubly deprotonated; b) singly protonated on the left oxygen; c) singly protonated on the middle oxygen; d) singly protonated on the right oxygen.

2. Computational Methods

The crystallographic structure of HIV RNase H was obtained the from PDB crystal structure 3K2P with bound β-thujaplicinol. Receptor preparation, including deprotonation and protonation states, was carried out with the software package Maestro m[5] using the force field OPLS_2005 [6]. The modeling simulation is carried out by replacing the manganese cations from the PDB crystal structure data base to native magnesium cations.

In the process of protein preparation, we cleared all the zero-order bonds to metals and delete all the water molecules before we get started. In addition, the three hydroxyl groups on the tropolone ring were modeled to be deprotonated as singly ionized and doubly deprotonated states within a pH range of 7.0±2.0. besides, we exploit the different positions on three oxygen atoms of tropolone ring: left, middle, right.

In order to investigate the presence and location of the hydrogen atom in detail, we adopted an accurate quantum mechanics method. As we involved a total number of 2068 of atoms, the complexity of the protein would produce extra cost to the system. We therefore introduced a Quantum Mechanics/ Molecular Mechanics (QM/MM) method to improve the modeling efficiency and lower the cost.[7] The definitions of the classical and Qm regions were carried out through QSite,[8] we chose the β-thujaplicinol, as well as the magnesium cations for the QM ligand/ion regions; we selected amino acid around the magnesium cations as side chains, which include ASP 549, ASP 443, GLU 478, ASP498 as belonging to the QM region. The QM region is updated before we run the jobs with 2 CPUs. All the quantum mechanical calculations were performed using Density Functional Theory (DFT) implementation in QSite with the functional of B3LYP in conjunction with lacvp* basis set.[9] And we choose 1000 maximum cycles for MM region; while 100 as the maximum iteration number for QM region. In order to mimic the

real biochemical atmosphere, we adopt distance dependent as electrostatic treatment, with a dielectric constant of 4.0.

3. Results

In order to figure out if the hydrogen protonation exists as well as where the protonation happens in the process of HIV inhibitor reacts with the receptor, we mimic the protonation process by introducing four different states of the ligand, doubly ionized β-thujaplicinol (2-), and three singly ionized β-thujaplicinol (1-) ligand with hydrogen protonated on the oxygen atoms of left, middle, right, respectively. By mapping the surface, we could visualize the binding site between the ligand and the receptor, which is shown in Figure 2.

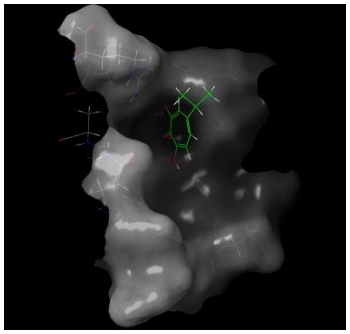

Figure 2. the binding site between ligand (green) and receptor(grey).

After QM/MM optimization, we found the structure of ligands in all four states shifted somewhat from the crystallographic position, as shown in the Figure 3. For reference, we adopt original crystallographic structure in green line from PDB database with hydrogen modifications, then all modelled structures shown in licorice mode.

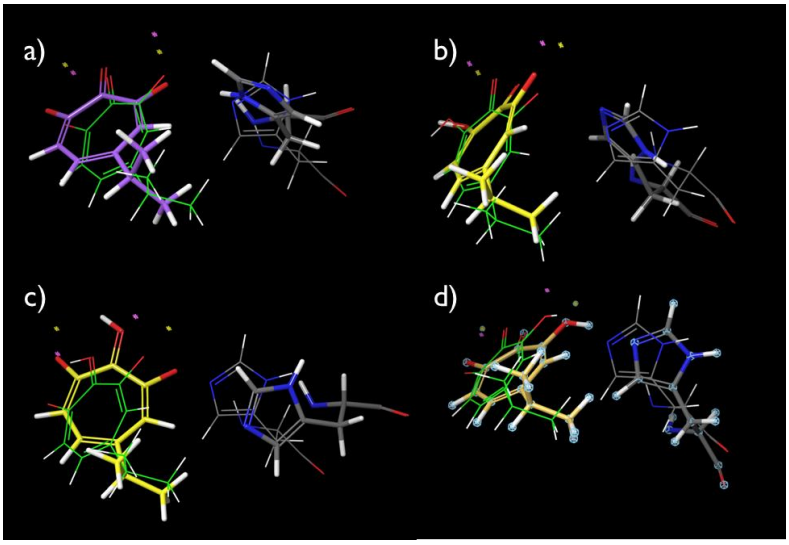

Figure 3. the structure comparison of calculated molecule to the original structure (the line structure with purple color Mg cations): (a) doubly deprotonated; (b) singly protonated on the left oxygen; (c) singly protonated on the middle oxygen; (d) singly protonated on the right oxygen.

Based on our results from the observations, listed in Table 1, the doubly ionized structure is most off relative the crystal structure, with tropolone rings moved 22 degrees while the histidine rings shifted 64 degrees. In contrast, the singly ionized structures did not sway much. However, when the hydrogen protonated on the middle oxygen, the structure has a ring angle shift of 27.8 degree from tropolone and 18.8 degree from histidine. The protonation positions occur on the left /right oxygen yielding very similar results. Right oxygen protonated molecule structure opens a small angle in one direction according to the distance measurement. The right oxygen protonation gives the closest structure to the crystallographic structure since all the three atoms are adjacent to the original positions smaller or close to 1 Å. In addition, the tropolone ring angle is a bit smaller than the left one. We also found that the relative position of magnesium cations shifted around 2 Å in all four cases. For the histidine shift, right positioned deprotonation yields the smallest distortion of 1.19 Å.

In conclusion, we proposed that the β-thujaplicinol with a left oxygen protonation structure is the ideal structure among those four as it could closely replicate the crystallographic structure.

Table 1. The angle changes of tropolone and histidine rings, as well as the distance (unit: Å) shifts.

	ring angles (tropolone)	ring anlgle(histidine)	left(o)	middle(o)	right(o)	Mg-Mg(left)	Mg-Mg(right)	histidine (head)
doubly	22	64	0.84	0.63	-0.66	0.64	1.48	2.97
singly (left)	17.8	51.1	-0.52	-0.62	0.61	1.04	0.95	2.13
singly (right)	14.8	7.9	-0.58	-0.87	-1.07	0.41	1.9	1.19
singly (middle)	27.8	18.8	-1.4	-1.12	-1.58	1.32	1.93	2.14

4. Future work

Based on our discovery, we also propose a following research topic of protonation positions to investigate histidine. There are three different states of hydrogen protonation, which include pros position, tele position, and both pros and tele positions, as shown in Figure 4.

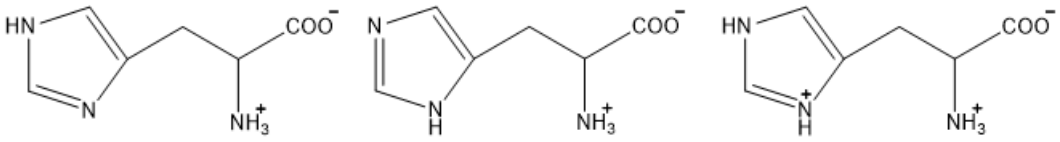

Figure 4. proposed protonation on histidine. Left: protonation on tele position; middle: protonation on pros position; right: protonation on both pros and tele positions.

To fully understand all the possible combinations, we total have 12 possibilities. After running the simulation with the method of QM/MM, the more closed molecular structure will be selected as the most likely protonation intermediate in the practical ligand and receptor reaction. Furthermore, we could expand our research method to other related topic, which could unveil the understanding of protonation mechanism in the ligand and receptor binding reaction, therefore provide instructive information to drug design and medical applications.

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