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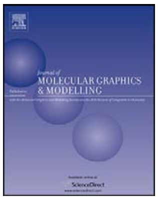

Bridge water mediates nevirapine binding to wild type and Y181C HIV-1 reverse transcriptase—Evidence from molecular dynamics simulations and MM-PBSA calculations

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ABSTRACT

The important role of the bridge water molecule in the binding of HIV-1 reverse transcriptase (RT) inhibitor complex was elucidated by molecular dynamics (MD) simulations using an MM-PBSA approach. Binding free energies and thermodynamic property differences for nevirapine bound to wild type and Y181C HIV-1 reverse transcriptase were investigated, and the results were compared with available experimental data. MD simulations over 3 ns revealed that the bridge water formed three characteristic hydrogen bonds to nevirapine and two residues, His235 and Leu234, in the binding pocket. The energetic derived model, which was determined from the consecutive addition of a water molecule, confirmed that only the contribution from the bridge water was essential in the binding configuration. Including this bridge water in the MM-PBSA calculations reoriented the binding energies from −32.20 to −37.65 kcal/mol and −28.07 to −29.82 kcal/mol in the wild type and Y181C HIV-1 RT, respectively. From the attractive interactions via the bridge water, His235 and Leu234 became major contributions. We found that the bridge water is the key in stabilizing the bound complex; however, in the Y181C RT complex this bridge water showed weaker hydrogen bond formation, lack of attractive force to nevirapine and lack of binding efficiency, leading to the failure of nevirapine against the Y181C HIV-1 RT. Moreover, the dynamics of Val179, Tyr181Cys, Gly190 and Leu234 in the binding pocket showed additional attractive energetic contributions in helping nevirapine binding. These findings that the presence of a water molecule in the hydrophobic binding site plays an important role are a step towards a quantitative understanding of the character of bridge water in enzyme-inhibitor binding. This can be helpful in developing designs for novel non-nucleoside HIV-1 RT inhibitors active against the mutant enzyme.

1. Introduction

Reverse transcriptase (RT) is a key enzyme in the replication cycle of the human immunodeficiency virus type 1 (HIV-1), catalyzing the conversion of virally encoded RNA into proviral DNA [1]. This essential step in the retroviral life cycle is targeted by a variety of drugs in clinical use to combat AIDS (acquired immune deficiency syndrome). RT is a heterodimeric enzyme with subunits of 66 and 51 kDa. The p66 subunit consists of fingers, palm and thumb subdomains (named for their resemblance to a right hand), as well as connection and ribonuclease H (RNase H) subdomains.

There are two main classes of RT inhibitors [2]. The first class consists of nucleoside analogues (nucleoside reverse transcriptase inhibitors, or NRTIs) such as AZT (3′-azido-3′-deoxythymidine), ddI (dideoxymosine) and ddC (dideoxycytidine). These are competitive inhibitors of the nucleotide substrate, and bind to the polymerase active site upon metabolic activation. After incorporation in the DNA strand instead of dNTP they cause premature termination of the newly synthesized chain. In addition, NRTIs also act on other host DNA polymerases, which explains their toxicity [3]. The second class consists of non-nucleoside inhibitors (NNRTIs) such as HEPT (1-[(2-hydroxyethoxy)-methyl]-6-(phenylthio)thymine) [4], TIBO (tetrahydroimidazo-[4,5,1-jk][1,4]-benzodiazepin-2(1H)-one) [5], nevirapine (dipyridodiazepinones) [6], and efavirenz ((−)-6-chloro-4-cyclopropyl ethynyl-4-trifluoromethyl-1,4-dihydro-2H-3,1-benzoxazin-2-one) [7]. These inhibitors are highly specific for HIV-1 RT and lock it into an inactive conformation by fitting into an allosteric site approximately 10 Å from the polymerase active site, causing a displacement of the catalytic aspartate residues. Furthermore, they show lower cellular toxicity than NRTIs.

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Reported crystal structures of RT include those of unliganded RT [8], RT complexed to dsDNA [9], RT bound to several NNRTIs [10,11], and RT complexed to dsDNA and a deoxynucleoside triphosphate (RT/dsDNA/dNTP) [12]. The non-nucleoside binding pocket only exists in the structures of RT complexed to a NNRTI, with its formation probably being induced by the proximity of the inhibitor. In unliganded RT, the p66 thumb subdomain is folded into the DNA-binding cleft and lies over the palm subdomain, nearly touching the fingers subdomain in a "thumb down" configuration. As a consequence, the DNA-binding cleft is closed. Moreover, the conformational change effected by NNRTI binding reduces the catalytic efficiency of the enzyme [13].

The rapidity of the selection of drug-resistant HIV in patients was such that single-point mutations in the virus made first-generation NNRTIs such as nevirapine unusable in monotherapy [14]. Among the mutations in RT that were originally described for nevirapine resistance were those at Tyr181 and Tyr188, both of which gave rise to high-level resistance [15]. The mutation of tyrosine at position 181 has frequently occurred, not only when nevirapine was treated but also in many other NNRTIs, and the change is almost always to cysteine. In the case of the mutation of tyrosine at position 188, a variety of mutation was reported. Nevirapine and HEPT select the Tyr188Cys mutation, whereas TIBO or α-APA result in Tyr188His or Tyr188Leu mutations [16]. Experimental results show that the non-nucleoside inhibitors lose their inhibitory efficiency by 20- to 1,000-fold when the mutation occurs in the HIV-1 RT binding pocket [17].

Computational modeling studies on HIV-1 RT and nevirapine have been performed for a few decades to gain more understanding at the molecular level. The conformational analysis of nevirapine by quantum calculation revealed that the cyclopropyl group is rotatable, but the minima appeared at 218° [18]. The basic postulate Gaussian network model (GNM) revealed that the p66 thumb's mobility was extremely sensitive when HIV-1 RT was bound to nevirapine. Collective motions analysis showed the key residues, Leu100, Trp229 and Leu234, covered nevirapine inside the binding pocket [19]. High-level quantum mechanical (QM) theory, implemented through the ONIOM approach, revealed weak π–π and H–π attractive interaction between Tyr181 and nevirapine [20,21]. Although the QM studies provide accurate interaction data according to theory, the studies based on rigid structures still lack information about the dynamics of binding in the RT-nevirapine bound complex. Thus, the simulations approach has also been applied to investigate the properties, interactions and binding energy of the reverse transcriptase complex to non-nucleoside inhibitors, in order to handle larger systems than can be accommodated by QM techniques [22]. The conformational changes of the RT pocket have been investigated through molecular dynamics (MD) simulations. Specific demonstration of the dihedral angle rotation of N-CA-CB-CG in Y181 showed the induced effect from mutation [23]. However, the interrelationship of nevirapine and the Y181C mutation also needs some proof from energetic prediction to be linked with the experimental affinity. The combined Monte Carlo (MC) simulations with free energy perturbation (FEP) revealed a fold resistance energy of 3.88 ± 0.3 kcal/mol from nevirapine relatively to efavirenz [15], and the effectiveness of nevirapine against the Y181C mutated form of the enzyme versus the wild type [24]. Ways to calculate the absolute binding free energy between HIV-1 RT and nevirapine have been developing since first being introduced with the QSAR correlation [25]. One of the most useful methods is the Molecular Mechanics/Poisson–Boltzmann Surface Area (MM-PBSA) method [26] because it is based on a compromise between speed and accuracy in the calculations. The combined MD/MM-PBSA method could explain the lost activity of nevirapine when the Y181C mutation occurs from the change in free energy by −5.94 kcal/mol [27]. Currently, numerous computational studies have shown quantitative agreement between calculated and experimental binding affinities in the HIV-1 RT system. However, the role of solvent molecules in the binding mechanism between the hydrophobic pocket and nevirapine is still of interest for further study.

Therefore, the main objective of this work was to study solvent molecules inside the hydrophobic binding pocket and introduce the key step in the MM-PBSA calculations when it was essential to include explicitly the solvent. The roles of the water molecule in HIV-1 RT/nevirapine complex have been reported previously by Rizzo and co-worker [28]. In this study we investigated a different water molecule, the bridge water molecule (WAT1067), which is located around the oxygen of nevirapine. A combination of molecular dynamics simulations and MM-PBSA calculations (MD/MM-PBSA) was applied to study the wild type and the Y181C HIV-1 RT/nevirapine complexes in order to obtain the theoretical binding energy, and observe the dynamics of the RT pocket. The motion and role of water inside the binding pocket were also investigated. The results will be useful in determining the molecular level of HIV-1 RT/nevirapine interaction in solution, and as guidance for other NNRTIs which have similar binding to nevirapine. This basic information can be used in the development of higher potency NNRTIs against mutant enzymes.

2. Computational methods

2.1. Molecular dynamics simulations

Model structures of the wild type and the Y181C HIV-1 RT complexes with nevirapine were constructed based on the crystallographic code 1VRT [29]. The enzymes were mutated at position 181 from tyrosine to cysteine by the SPdbV3.7 program [49]. After all missing residues were added, the mutant model was then minimized using 5,000 steps of steepest descent, and then switched to the conjugate gradient algorithm in the Sander module of the AMBER program package in order to remove bad steric interactions. Nevirapine was firstly optimized at the B3LYP/6-31G(d,p) level. Then the electrostatic potential that surrounds nevirapine was calculated by a single-point calculation at the HF/6-31G(d) level in GAUSSIAN98 [30]. The electrostatic potential was fitted into the partial atomic charge of nevirapine by the RESP [31,32] charge method. Preparation of the force field parameters of nevirapine was done by using the Antechamber module [33] of AMBER.

Molecular dynamics simulations were performed using an AMBER7 [34] program with an AMBER 1999 force field [35]. Each complex system was immersed in an octahedral box of 10 Å from the solute surface using TIP3P water [36]. A total of 42,170 solvent molecules were generated followed by 7 Cl^- ions for neutralization, resulting in a total of 143,900 atoms in the system. Periodic boundary conditions and constant temperature and pressure were used. The non-bonded cutoff distance was set to 11.5 Å. The integration time step was 2 fs, with SHAKE [37] applied to constrain the bonds involving hydrogen atoms. The restraint force on the whole complex was slowly decreased from 4.0 kcal/mol to zero during the equilibration, and simulations were run for 1 ns at 300 K. Coordinates were saved every 1 ps. After the entire HIV-1 RT/nevirapine system had been simulated for 1 ns, the low energy structure at the equilibrium was selected as the next starting structure. In order to speed up the calculation, the structure of the enzyme complexes was reduced to a smaller model in which protein residues and water molecules outside 30 Å of the mass center of nevirapine were removed. Continuation of the simulations was performed by using the AMBER9 [38] program with the Duan et al. (2003) force field [39,40] which provided a better force field for proteins. The model was set up as follows: all water