Archives of Biochemistry and Biophysics 635 (2017) 87–95

Contents lists available at ScienceDirect

Archives of Biochemistry and Biophysics

journal homepage: www.elsevier.com/locate/yabbi

Oxygen binding isotope effects of triazole-based HIV-1 reverse transcriptase inhibitors indicate the actual binding site

Agnieszka Krzemińska, Tomasz Frączek, P. Paneth*

Institute of Applied Radiation Chemistry, Lodz University of Technology, 90-924 Lodz, Poland

ARTICLE INFO

Keywords:
HIV-1 reverse transcriptase
Binding isotope effects
QM/MM
FEP
Free energy of binding
Interaction energy
Potential energy
NNRTIs
HAART
Triazole-based RTIs

ABSTRACT

Binding isotope effects (BIEs) associated with binding of four triazole-based ligands to HIV-1 reverse transcriptase have been calculated at the QM/MM MD level of theory. Two main binding sites: allosteric cavity and RNase H active site, as well as three other sites reported in the literature (the Knuckles, the NNRTI Adjacent, and Incoming Nucleotide Binding) have been considered. The interactions between inhibitors and these protein sites have been quantified by binding free energies obtained from free energy perturbation (FEP) calculations, supported by interaction energy analysis. It has been shown that binding in the allosteric cavity can be distinguished from binding to other sites based on BIEs as it is associated with normal ^{18}O-BIEs of the carbonyl oxygen atom while binding to RNase H active site is characterized by inverse binding isotope effect (^{18}O-BIE < 1). For other sites ^{18}O-BIEs close to unity are predicted. This information points to oxygen binding isotope effects of carbonyl group as indicative of the actual binding site of studied inhibitors.

1. Introduction

Since the 11th International Conference on acquired immune deficiency syndrome (AIDS) held in Vancouver in 1996, "highly active antiretroviral therapy" (HAART)–a cocktail of different drugs is used in treatment of Human Immunodeficiency Virus (HIV) infected patients [1]. According to the World Health Organization (WHO) data AIDS mortality after introduction of the HAART has been reduced by approximately 60% [2]. However, due to undesired side-effects, especially occurring during long-term and multi-drug treatments and drug-resistance caused by HIV-1 enzymes mutations, there is still considerable interest in discovering new antivirals. One of the drug targets is HIV-1 Reverse Transcriptase (RT), an essential 117 kDa enzyme needed in transcription of single-strand viral RNA into viral DNA double helix. RT is a heterodimer composed of p66 and p51 subunits. The larger 560 amino acids p66 subunit provides enzymatic activity, and has thumb, palm, fingers and RNase H domains, as illustrated in Fig. 1. The enzyme contains one DNA polymerization active site, and one ribonuclease H (RNase H) active site, both of which reside in the p66 subunit at spatially distinct regions [3,4]. Half of 26 approved antiretroviral drugs target polymerase active site of RT or the allosteric cavity located 10 Å away. Five of them are non-nucleoside RT inhibitors (NNRTIs) which bind in the allosteric cavity, inhibiting the enzyme via molecular wedge mechanism [5]. Nucleoside RT inhibitors (NRTIs) compete with natural substrates causing viral DNA chain termination, but they may induce toxicity by affecting human host polymerases [6]. Recently, new inhibitors targeting the RNase H activity of RT by combining nucleoside (NRTIs) and non-nucleoside inhibitors (NNRTIs) are also being developed [5]. Moreover, fragment screening by X-ray crystallography showed that RT has also seven other sites (see Fig. 1.) capable of binding inhibitors: the Incoming Nucleotide Binding, Knuckles, NNRTI Adjacent, the 399 site from the polymerase region, the 428 site, RNase H Primer Grip Adjacent, and the 507 site from RNase H region [7]. Only three of these: the Knuckles, the NNRTI Adjacent, and the Incoming Nucleotide Binding exhibit potent inhibitory activity in RT assays. Accurate description of ligand-enzyme binding affinity plays an essential role in rational drug design, but it is a challenging task, especially when ligand can bind to a few different binding sites. Measured values constitute average affinities of different binding sites. In order to describe ligand-pocket interactions explicitly for different sites, theoretical tools are required. The most sensitive among them, and used frequently in many fields of chemistry and biology, are isotope effects (IEs) [8]. Quantitatively, IEs report on changes in the strength of bonds to the isotopic atom. When the bonding environment is weaker in the products than in the reactants (i.e., weaker bonds, or fewer number of bonds) an isotope effect is larger than unity (normal IE). In the opposite case the IE is less than unity (inverse isotope effect). Upon ligand binding previously flexible groups are immobilized; polarization of bonds, as well as the geometry change. These effects generate observable binding IEs (BIEs). When bonding environment of a given atom

* Corresponding author.
E-mail address: paneth@p.lodz.pl (P. Paneth).

http://dx.doi.org/10.1016/j.abb.2017.10.017
Received 25 July 2017; Received in revised form 4 October 2017; Accepted 24 October 2017
Available online 27 October 2017
0003-9861/

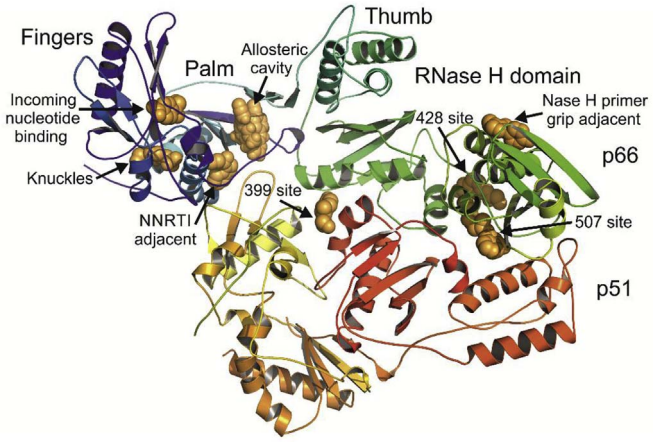

Fig. 1. Structure of HIV-1 RT heterodimer with highlighted thumb, palm, fingers and RNase H domains from p66 subunit and indicated seven allosteric binding sites: the Incoming Nucleotide Binding, Knuckles, NNRTI Adjacent, the 399 site from polymerase region of RT, the 428 site, RNase H Primer Grip Adjacent, the 507 site from RNase H region.

of an inhibitor is weaker in the protein than in the solution, normal BIEs are observed. If, however, bonding environment is tighter in the inhibitor-protein complex than interactions of the inhibitor with the solvent molecules in the solution, BIE < 1. Thus BIEs allow to elucidate inhibitor-protein interactions [9].

We have successfully used [10–12] heavy-atoms BIEs calculations to get insight into inhibitory processes. In theoretical [12] and experimental [13] studies we have recently shown that N-(2-chloro-4-sulphamoylphenyl)-2-((4-(2,4-dimethyl-phenyl)-5-(thiophen-2-yl)-4H-1,2,4-triazol-3-yl)sulphenyl)-acetamide, L-1, is an inhibitor of HIV-1 RT that presumably binds in the hydrophobic allosteric pocket via van de Waals interactions, as observed for NNRTIs. However, L-1 also binds to the RNase H active site. Promising properties obtained for L-1 prompted us to test its analogues depicted in Fig. 2. The sulphonamide group of L-1 was replaced by carboxyl group, in L-2 and L-3, or amide group in L-4 case. Additionally, thiophene ring was replaced by methyl group in L-3. In this contribution we explore applicability of BIEs to identification of the actual binding site with aim of finding the ligand which should exhibit the largest difference in BIEs between the binding sites. To this end we have extended our previous studies of L-1 to three other compounds (L2–L-4), and studied their binding in both allosteric cavity and the RNase H active site. Subsequently, for L-3 the comparison was extended to three other binding sites: the Knuckles, the NNRTI Adjacent, and the Incoming Nucleotide Binding; the free energy of binding of L-3 to different binding sites have been computed using FEP method.

2. Materials and methods

2.1. System setup

The initial coordinates of HIV-1 RT were taken from the Protein Data Bank, entries with PDB ID: 2RKI [14] with 4-benzyl-3-[(2-chlorobenzyl)sulphanyl]-5-thiophen-2-yl-4H-1,2,4-triazole (TT1) ligand bound in the allosteric cavity; 4IFY [7] structure with ligand 4-{[4-({4-[(E)-2-cyanoethenyl]-2,6-dimethylphenyl}amino)pyrimidin-2-yl] amino}benzonitrile (T27) bound to Knuckles; 4ICL [7] with ligand T27 bound to Incoming Nucleotide Binding; and 4KFB [7] with T27 ligand bound to NNRTI Adjacent binding site. Side-chain atoms were checked using SCit tool, available online (http://bioserv.rpbs.jussieu.fr/cgi-bin/SCit) [15]. Missing hydrogen atoms were added with tLEAP from AmberTools ver. 12 [16] with the pKa values for titratable amino acids calculated with PROPKA software ver. 3.0 [17–19]. Based on native ligands arrangement in 2RKI, 4ICL, 4IFY, and 4KFB, novel four triazole N-(2-chloro-4-sulphamoylphenyl)-2-((4-(2,4-dimethylphenyl)-5-(thiophen-2-yl)-4H-1,2,4-triazol-3yl)sulphanyl acetamide derivatives illustrated in Fig. 2, have been docked using Glide package implemented in the Schrödinger program [20]. Four triazole derivatives (L-1, L-2, L-3, and L-4) were docked to the allosteric cavity and RNase H active site, while L-1 and L-3 were also docked to Knuckles, Incoming Nucleotide Binding, and NNRTI Adjacent binding sites. Crystallographic structures from PDB were prepared using Protein Preparation Wizard in Maestro [21]. Preprocessing step was performed with default settings, all water molecules and ions were removed, hydrogen bond network was optimized, followed by global optimization of all atoms using OPLS2005 force field. The resulting structures were used to prepare receptor grids. The sizes of grid boxes were kept default and centred on native ligands from crystallographic structures. Van der Waals radii of atoms with partial charges less than 0.2 were scaled down by a factor of 0.9. The remaining settings were kept default. Ligands for docking were prepared using the LigPrep utility [22]. The first stage of docking was carried out using Glide Standard Precision protocol [23–25] with default settings. Three best poses for each ligand were kept, then one best pose was selected, and the ligand-protein complex was subjected to MD simulations. In the second stage of docking, receptor grids were

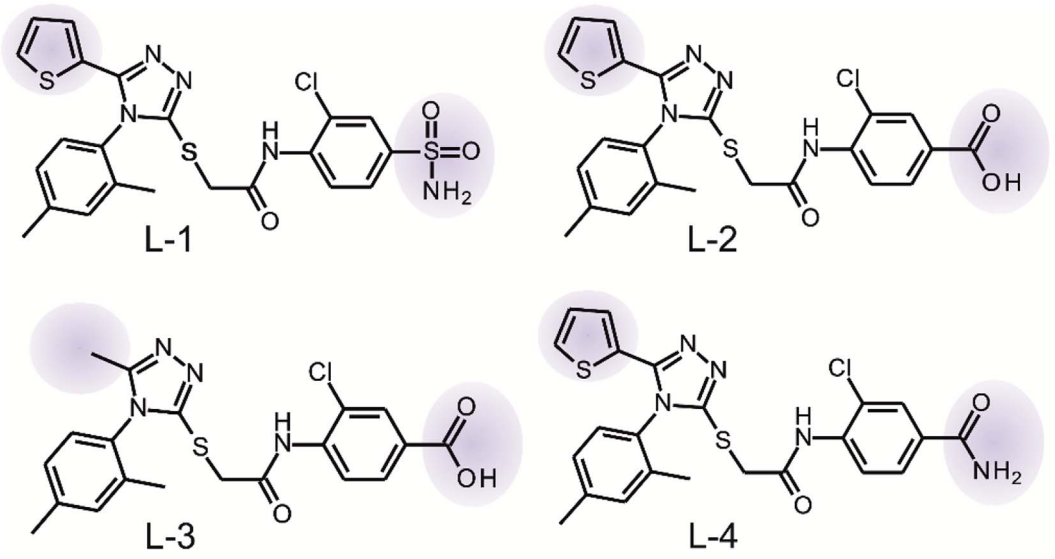

Fig. 2. Structure of N-(2-chloro-4-sulphamoylphenyl)-2-((4-(2,4-dimethyl-phenyl)-5-(thiophen-2-yl)-4H-1,2,4-triazol-3-yl)sulphenyl)-acetamide, L-1, and its three studied analogues.