ONIOM DFT/PM3 calculations on the interaction between dapivirine and HIV-1 reverse transcriptase, a theoretical study

Yong-Hong Liang, Fen-Er Chen*

Department of Chemistry, Fudan University, Shanghai, China.

ABSTRACT: Theoretical investigations of the interaction between dapivirine and the HIV-1 RT binding site have been performed by the ONIOM2 (B3LYP/6-31G (d,p): PM3) and B3LYP/6-31G (d,p) methods. The results derived from this study indicate that this inhibitor dapivirine forms two hydrogen bonds with Lys101 and exhibits strong π-π stacking or H…π interaction with Tyr181 and Tyr188. These interactions play a vital role in stabilizing the NNIBP/dapivirine complex. Additionally, the predicted binding energy of the BBF optimized structure for this complex system is -18.20 kcal/mol.

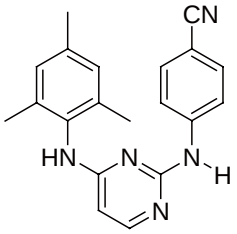
Key Words: Dapivirine, HIV-1 reverse transcriptase, ONIOM, DFT, PM3

Introduction

Human immunodeficiency virus type-1 reverse transcriptase (HIV-1 RT) is an important target for designing RT inhibitors to block the virus's replication and prevent AIDS-related disease (*1*,*2*). Two kinds of RT inhibitors, nucleoside reverse transcriptase inhibitors (NRTIs) and non-nucleoside reverse transcriptase inhibitors (NNRTIs), have been developed over the past twenty years (*3*). Despite NNRTIs such as three FDA-approved drugs, nevirapine (*4*), delavirdine (*5*) and efavirenz (*6*), being highly specific and less toxic than nucleoside inhibitors (*7*), the rapid emergence of resistant HIV viral strains carrying mutation at residues that surround the NNRTI binding pocket limits the usefulness of NNRTI to treat HIV infection (*8*).

Dapivirine (Figure 1), an early compound of NNRTIs diarylpyrimidines (DAPYs) (*9*,*10*), is currently in phase IIB clinical trials in the United States as an RT inhibitor for the treatment of AIDS. Unlike the butterfly-like conformation of nevirapine, delavirdine, and efavirenz in the crystal structures of HIV-1 RT/NNRTI complexes, this inhibitor adopts the horseshoe mode to bind with HIV-1 RT (*12*). Despite intensive experimental research including crystal structure analysis to study the interaction between dapivirine and HIV-1 RT (*11*,*12*), the interaction of dapivirine and amino acid in the non-nucleoside inhibitor binding pocket (NNIBP) and the origin of mutational effects are still not fully understood.

In the present work, the interaction between dapivirine and RT binding sites has been investigated by the ONIOM2 (B3LYP/6-31G (d,p): PM3) and B3LYP/6-31G (d,p) methods. The main aim of this work is to study the following two aspects: 1) the binding mechanism of dapivirine to HIV-1 RT and the nature of drug resistance and 2) the differentia of dapivirine with respect to two other inhibitors studied, nevirapine and efavirenz.



1 dapirivine (TMC 120)

Figure 1. The chemical structure of dapivirine.

Materials and Methods

Construction of the model studied

The model of NNIBP/dapivirine complex has been constructed as in previous reports (*13*,*15*). The binding pocket studied, which contains 19 residues surrounding the NNIBP with at least one atom interacting with any of the atoms of the inhibitor within an interatomic distance of 6 Å (Figure 2), is extracted from the 2.9 Å resolved crystal structure of dapivirine with HIV-1 RT (1S6Q) (*12*). All residues, supposedly in their neutral from, were terminated if not connected to another

**Correspondence to:* Department of Chemistry, Fudan University, Shanghai 200433, China;
e-mail: rfchen@fudan.edu.cn

Received June 9, 2007
Accepted June 20, 2007

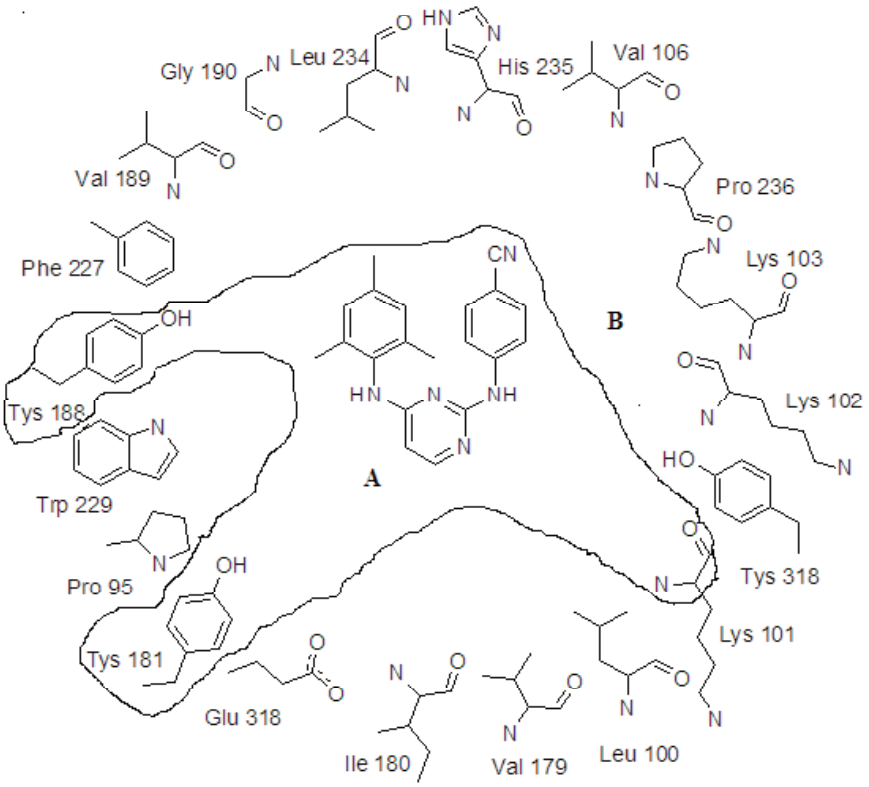


Figure 2. The studied model system of dapivirine bound to the HIV-1 RT binding site. Layer partition is shown for ONIOM2 calculations. (A) is the inner layer and (B) is the outer layer.

residue in the selected model by linking H atoms at the N- and C-terminal with their torsion angles, which are assumed to be the same as in the crystal structure. Hydrogen atoms were added to using the Gauss View program, and their positions were optimized by the semi-empirical PM3 method. This complex was used as the initial structure for this theoretical study.

ONIOM calculations

The ONIOM method was proposed as a reliable tool for studying enzyme-inhibitor interaction (*13-16*). Recently, Morokuma *et al.* (*17*) have systematically investigated all possible three- and two-layer ONIOM combinations of high-level QM (HQ = B3LYP/6-31G (d), low-level QM (LQ = AM1), and MM (Amber) for the deprotonation energy and structure of a zwitterionic peptide molecule, NH_3^+-CH^nBu-CO-NH-CH_2-CO-NH-CH^nBu-COO^-. They found that the errors introduced in the ONIOM approximation, in comparison to the target HQ (or HQ: HQ: HQ) calculation, generally increase in the following order: HQ: HQ: HQ (target) < HQ: HQ: LQ < HQ: LQ: LQ < HQ: HQ: MM < HQ: LQ: MM, HQ: MM: MM, LQ: LQ: LQ < LQ: LQ: MM < LQ: MM: MM. Thus, the two-layer ONIOM (B3LYP/6-31G (d,p): PM3) method is applied to calculate the structure and binding energy of dapivirine at the HIV-RT binding site. Previous reports (*11,12*) indicated that dapivirine has two strong binding sites with HIV-RT--the π-π interaction with aromatic rings of Tyr181 and Tyr188 and the hydrogen bond interaction with Lys101, so dapivirine was selected with two aromatic rings of Tyr181 and Tyr188 and Lys101 as the inner layer (Figure 2).

Optimizations have been performed while considering two approximations, heavy atoms fixing (HAF) and backbone atoms fixing (BBF). The binding energies of dapivirine with individual residues are calculated at the B3LYP/6-31G (d,p) level with correction for the basis set superposition error (BSSE), using the Boys-Bernardi counterpoise technique (*18*). All calculations presented here have been performed with the Gaussian 03 series of programs (*19*).

Results and Discussion

Structure and binding energy of HIV-1 RT binding site/ dapivirine

The main purpose of this work was to study the interaction between dapivirine and the binding sites of HIV-1 RT. Presented first is a discussion of the interaction of dapivirine with individual residues around the NNIBP. As depicted in Figure 3A, Lys101 is found to form two hydrogen bonds with 2-aminopyrimidine in the middle part of dapivirine; one is the nitrogen of the pyrimidine ring with the amino hydrogen of Lys101 and the other H-bond is the amino hydrogen of 2-aminopyrimidine with the backbone carbonyl oxygen of Lys101. Additionally, the distances of two hydrogen bonds in the X-ray structure are 3.51 and 2.75 Å for N…H-N and N-H…

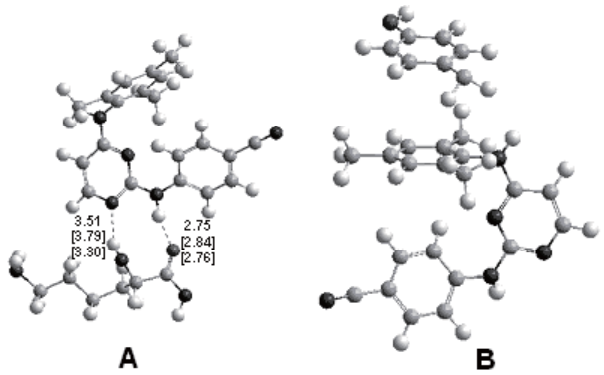


Figure 3. Optimized structure of the dapivirine and Lys101 (A), and Tyr181 (B) complex from ONIOM (B3LYP/6-31G (d,p): PM3).

O, respectively. The predicted H-bond distances of N…H-N and N-H…O in the BBF optimized geometries are consistent with the experimental results (3.38 and 2.76 Å, respectively). However, the H-bond distances in the HAF optimized geometries are predicted to lengthen by 0.28 and 0.09 Å, respectively. Meanwhile, the amino linkages of the two aryl rings provide sufficient flexibility to allow favorable π-π interaction with Tyr181, Tyr188, Trp229 and Tyr318; the aryl ring in the left moiety of dapivirine and the aryl ring of Tyr181 form a strong π-π stacking interaction, and two benzene rings are in parallel as shown in Figure 3B.

Table 1 shows the BSSE-corrected energies of the interaction of dapivirine with individual residues surrounding the NNIBP at the B3LYP/6-31G (d,p) level of theory. The results clearly deny that more attractive interactions are found. The interactions of dapivirine with all residues stabilize the NNIBP/dapivirine complex, and the interaction energies for the X-ray structure range from -0.6 to -4.1 kcal/mol. But the interaction of dapivirine with Leu100, Lys101, Lys103, Tyr181 and Tyr188 are the main contributors to stabilization of the dapivirine/NNIBP complex, and the interaction energies in the X-ray structure are -2.86, -2.06, -4.21, -3.16 and -2.10 kcal/mol, respectively. The calculated interaction energies obtained by the HAF and BBF optimized structures are consistent with those of the X-ray structure. These results are also consistent with the experimental results that the mutations of Leu100Ile, Tyr181Cys, Tyr188Leu and Leu103Asn+Tyr181Cys give rise to drug resistance to dapivirine (*9*). The Leu100Ile and Leu103Asn mutations generally destabilized the complex by diminishing the hydrogen bonds between the inhibitor and Leu101. Additionally, the mutations of Tyr181Cys and Tyr188Leu had resistance to dapivirine by reducing favorable π-π interaction between the tyrosine and aromatic rings of dapivirine.

As the dapivirine/NNIBP complex system is too large for high level calculations, ONIOM2 methods were thus adopted in order to calculate the binding energy of the dapivirine/NNIBP complex. This complex system is divided into two parts: the inner layer,

Table 1. BSSE-corrected interaction energies of dapivirine with individual residues (X_i) (in kcal/mol), calculated at the B3LYP/6-31G (d,p) level

Residue	Crystal	HAF	BBF
Pro95	-0.39	-0.50	-0.54
Leu100	-2.86	-2.64	-2.37
Lys101	-4.21	-4.12	-4.45
Lys102	-0.40	-0.25	-0.24
Lys103	-2.06	-1.94	-1.33
Val106	-0.98	-0.70	-0.73
Val179	-1.65	-1.41	-1.10
Ile180	-0.29	-0.19	-0.28
Tyr181	-3.16	-2.80	-2.37
Tyr188	-2.10	-1.34	-1.08
Val189	-0.31	-0.19	-0.14
Gly190	-0.32	-0.08	-0.09
Phe227	-1.18	-0.36	-0.63
Trp229	-0.83	-0.96	-1.37
Leu234	-1.71	-1.47	-1.37
His235	-1.08	-1.16	-0.84
Pro236	-0.65	-0.93	-0.69
Tyr318	-0.93	-0.66	-0.14
Glu138	-0.62	-0.88	-1.63
Total	-25.76	-22.58	-21.41

Table 2. Binding energies (BE kcal/mol) and their components for the HIV-1 RT/dapivirine complex, calculated by the ONIOM (B3LYP/6-31G (d,p): PM3) method

Method	BE	IE	DE	DE_{TMC120}	DE_{pocket}
HAF	-7.97	-13.95	5.98	3.67	2.31
BBF	-18.20	-23.11	4.91	4.58	0.33

consisting of dapivirine, Lys101, and two aromatic rings of Tyr181 and Tyr188, and the outer layer, consisting of the remaining residues. The B3LYP/6-31G (d,p) method has been proposed as a reliable tool for studying molecular systems containing intermolecular hydrogen bonds (*20*), so this method is applied to the inner layer because the hydrogen bond interaction between dapivirine and Lys101 is the main contributor. The calculated ONIOM2 binding energies (BE), interaction energies (IE), and deformation energies (DE) for the dapivirine/NNIBP complex are shown in Table 2. The binding energy for BBF was found to be -18.20 kcal/mol, while the results obtained by HAF underestimated the binding energy by 10.23 kcal/mol.

Comparison of dapivirine, efavirenz, and nevirapine bound to HIV-1 RT

Upon comparison of two NNRTI drugs studied, efavirenz and nevirapine (*13,14*), dapivirine was found to have the virtues of two inhibitors. Based on previous reports, efavirenz has two strong hydrogen bonds with Lys101 and the aromatic pyridine ring of nevirapine has strong π-π stacking or H…π interaction with two aromatic rings of Tyr181 and Tyr188. In the structure of dapivirine, the 2-aminopyrimidine groups of dapivirine, which are equivalent to the benzoxazin-2-one in efavirenz, form two hydrogen bonds with the carbonyl oxygen and amino hydrogen of Lys101, and the

aromatic ring in the left moiety of dapivirine exhibits strong π-π stacking or H…π interaction with the two aromatic rings of Tyr181 and Tyr188. The calculated interaction energies of dapivirine with all residues stabilize the dapivirine/NNIBP complex; but with some residues the interaction energies of efavirenz and nevirapine destabilize the inhibitor/NNIBP complex.

Conclusions

ONIOM calculations of the NNIBP/dapivirine complexes systems show that dapivirine has strong interaction with NNIBP, and the predicted binding energies of the NNIBP/dapivirine complex are respectively -7.97 and -18.2 kcal/mol for HAF and BBF by the ONIOM2 (B3LYP/6-31G (d,p): PM3) method. The 2-aminopyrimidine groups of dapivirine form two hydrogen bonds with the carbonyl oxygen and amino hydrogen of Lys101, and two aromatic residues, Tyr181 and Tyr188, are found to exhibit strong H… π and π-π interaction with the aromatic ring in the left moiety of dapivirine. These distinctive characteristics of dapivirine binding to NNIBP play a vital role in stabilizing the complex. Therefore, dapivirine is obviously in line to become a new generation of inhibitor to combat AIDS.

Acknowledgements

This work was supported by grants from the National Natural Science Foundation of China (No: 30672536).

References

1. De Clercq E. Strategies in the design of antiviral drugs. Nat Rev Drug Discov 2002;26:13-25.
2. Sarafianos SG, Das K, Lewi PJ, Hughes SH, Arnold E. Taking aim at a moving target: design drugs to inhibit drug-resistant HIV-1 reverse transcriptase. Curr Opin Struct Biol 2004;14:716-730.
3. Jonckheere H, Anné J, De Clercq E. The HIV-1 reverse transcriptase (RT) process as target for RT inhibitors. Med Res Rev 2000;20:129-154.
4. Merluzzi VJ, Hargrave KD, Labadia M, Grozinger K, Skoog M, Wu JC, Shih CK, Eckner K, Hattox S, Adams J. Inhibition of HIV-1 replication by a nonnucleoside HIV-1 reverse transcriptase inhibitor. Science 1990;250:1411-1413.
5. Freimuth WW. Delavirdine mesylate, a potent nonnucleoside HIV-1 reverse transcriptase inhibitor. Adv Exp Med Biol 1996;394:279-289.
6. Young SD, Britcher SF, Tran LO, *et al*. L-743726 (DMP-266): a novel highly potent nonnucleoside inhibitor of the human immunodeficiency virus type 1 reverse transcriptase. Antimicrob Agents Chemother 1995;39:2602-2605.
7. Schinazi RF, Mead JR, Feorino PM. Insights into HIV chemotherapy. AIDS Res Hum Retroviruses 1992;8:963-990.
8. Richman DD, HIV drug resistance. AIDS Res Hum Retroviruses 1992;8:1065-1071.
9. Ludovici DW, De Corte BL, Kukla MJ, *et al*. Evolution of anti-HIV drug candidates. Part 3: diarylpyrimidine (DAPY) analogues. Bioorg Med Chem Lett 2001;11:2235-2239.
10. Guillemont J, Pasquier E, Palandjian P, *et al*. Synthesis of novel diarylpyrimidine analogues and their antiviral activity against human immunodeficiency virus type 1. J Med Chem 2005;48:2072-2072.
11. Das K, Lewi PJ, Hughes SH, Arnold E. Crystallography and the design of anti-AIDS drugs: conformational flexibility and positional adaptability are important in the design of non-nucleoside HIV-1 reverse transcriptase inhibitors. Prog Biophys Mol Biol 2005;8:209-231.
12. Das K, Clark AD, Lewi PJ, *et al*. Roles of conformational and positional adaptability in structure-based design of TMC125-R165335 (etravirine) and related non-nucleoside reverse transcriptase inhibitors that are highly potent and effective against wild-type and drug-resistant HIV-1 variants. J Med Chem 2004;47:2550-2560.
13. Kuno M, Hannongbua S, Morokuma K. Theoretical investigation on nevirapine and HIV-1 reverse transcriptase binding site interaction, based on ONIOM method. Chem Phys Lett 2003;380:456-463.
14. Nunrium P, Kuno M, Saen-oon S, Hannongbua S. Particular interaction between efavirenz and the HIV-1 reverse transcriptase binding site as explained by the ONIOM2 method. Chem Phys Lett 2005;405:198-202.
15. Yao LS, Cukier RI, Yan HG. Catalytic mechanism of guanine deaminase: an ONIOM and molecular dynamics study. J Phys Chem B 2007;111:4200-4210.
16. Majumdar D, Roszak S, Leszczynski J. Probing the acetylcholinesterase inhibition of sarin: A comparative interaction study of the inhibitor and acetylcholine with a model enzyme cavity. J Phys Chem B 2006;110:13597-13607.
17. Morokuma K, Wang Q, Vreven T. Performance evaluation of the three-layer ONIOM method: case study for a zwitterionic peptide. J Chem Theor Comput 2006;2:1317-1324.
18. Boys SF, Bernardi F. The calculation of small molecular interactions by the differences of separate total energies, some procedures with reduced errors. Mol Phys 1970;19:553-566.
19. Frisch MJ, Trucks GW, Schlegel HB, *et al*. Gaussian 03 Revision B.03, Gaussian, Inc., Pittsburgh PA, 2003.
20. Yi PG, Liang YH, Tang ZQ. Theoretical study of intermolecular proton transfer reaction in isolated 5-hydroxyisoxazole--water complexes. Chem Phys 2006;322:387-391.