Investigation on an Orientation and Interaction Energy of the Water Molecule in the HIV-1 Reverse Transcriptase Active Site by Quantum Chemical Calculations

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Received October 2, 2002

To obtain basic information such as interaction between the water molecule and amino acids in the active site of HIV-1 Reverse Transcriptase (HIV-1 RT), ab initio molecular orbital calculations and the two-layer ONIOM method were performed. The energetic results from different methods show that the ONIOM2 (MP2/6-311G**:HF/6-31G*//HF/6-31G**:HF/3-21G) can provide reliable results on the orientation of the water molecule in the HIV-1 RT active site. The interaction between the water molecule and Asp186 was found to be the most preferable. The obtained results from ONIOM2 calculations indicated that the active site model system included six amino acid residues (Asp186, Asp185, Met184, Tyr183, Leu187, and Tyr188) leading a preferable representation of the environment surrounding the water molecule in the more realistic model. The water molecule presented in the active site tends to form H-bonding with Asp186, Tyr183, and Tyr188 as indicated by the distances of O4−H2 = 1.91 Å, O3−H7 = 2.36 Å, and O3−H17 = 1.73 Å, respectively. The stability of this complex system brings to the foundation of the estimated binding energy approximately −15.8 kcal/mol or −8.1 kcal/mol which is more stabilized relative to the smallest model complex. These observations revealed that the water molecule forms both a hydrogen bond donor and a hydrogen bond acceptor in the cavity and plays an important role in the specific conformation of the active site of HIV-1 RT. The H-bonding is a rather strong interaction; thus, the water might induce the conformation of the active site to fit the catalysis process and helpfully attract dNTP to elongate the viral DNA in the replication process of this enzyme.

1. INTRODUCTION

HIV-1 reverse transcriptase (HIV-1 RT) is known as the causative agent of acquired immunodeficiency syndrome (AIDS) and is an essential enzyme involved in the life cycle of the HIV virus responsible for copying the single-strand RNA viral genome into a double-stranded proviral DNA form for integration into the host genome. Therefore, it has been an important target of several antiviral therapeutic agents used in the treatment of AIDS.[1−4] HIV-1 RT inhibitors have been developed and classified into two main categories: nucleoside and non-nucleoside analogues.[5−11] The nucleoside RT inhibitors (NRTIs) such as AZT, ddC, and ddI have been widely used to treat AIDS patients. Unfortunately, these derivatives have suffered from loss of antiviral activity, high cellular toxicity, several side effects, and the emergence of drug-resistant viral variants.[5,7,13,14] The other class of HIV-1 RT inhibitors is the non-nucleosides (NNRTIs) such as HEPT,[15] TIBO,[16] nevirapine,[17] and efavirenz,[18] which are highly specific for HIV-1 RT. The NNRTIs are much less toxic than the NRTIs; nevertheless, the emergence of drug-resistant viral strains has limited the therapeutic efficacy of NNRTIs.[6,7,9,10,12,19]

To date, the development of X-ray crystallography has provided more than 50 valuable crystal structures of HIV-1 RT, which are available in the Protein Data Bank (RCSB PDB, http://www.rcsb.org).[20] The catalytic site for RT lies in a cleft in the palm of the p66 subunit and contains the sequence Tyr183, Met184, Asp185, and Asp186, which are highly conserved in retroviral RTs as well as in other DNA polymerase. Asp110 is also required for catalytic activity and is brought into the active site in the folded protein. The 3′-OH of the primer terminus is held close to the catalytically essential Asp110, Asp185, Asp186 residues; thus, this terminus is in a position for nucleophilic attack on the α-phosphate of an incoming nucleoside triphosphate.[5,11] Observation of a hydrogen bond between the 3′-OH of the primer terminus and the side chain of Asp185 suggests that the carboxylate of Asp185 could act as a general base in initiating the nucleophilic attack during polymerization.[21] It was reported that the polymerase of HIV-1 RT requires divalent metal ions (preferably Mg^{2+}) as cofactors and proposed that there are two Mg^{2+} bound to the active site aspartate residues in the active form of HIV-1 RT.[21] Interaction between proximal oxygen atoms of Asp186 and the 3′-terminal phosphate may be mediated via either a metal ion or a water molecule. Such a water molecule might also be the ligand for one metal ion at the polymerase active site.[22] Evidently, there are 27 crystal structures of HIV-1 RT complex with the existence of the water molecules in the polymerase active site cavity. It is questioned how important these water molecules are in the polymerase active site. Based on ab initio Molecular Dynamics Simulations[23] study on HIV-1 RT, it was shown that water molecules present in the active site may be crucial for substrate recognition. Therefore, theoretical investigation can be used to obtain the basic information of the role of the water molecule and the interaction with the polymerase active site of HIV-1 RT.

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10.1021/ci0203850 CCC: $25.00
Published on Web 07/04/2003

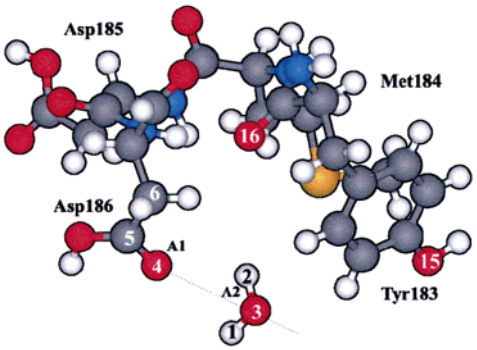

Figure 1. The starting geometry of HIV-1 RT/water complex used in this study (the arrow indicates the direction of translation for the single point calculations scanning at the HF/3-21G level).

Recently, the multilayered integration method or ONIOM (Our own N-layered Integrated molecular Orbital + molecular Mechanics) has been introduced[24] and has been proven to be a powerful tool for the theoretical treatment of large molecular systems where different levels of theory are applied to different parts of the molecule.[25−28] The most important part of the molecule forms the innermost layer described at the highest level of theory. Subsequent layers are treated by using progressively computationally cheaper lower-level approaches. In this study, ab initio quantum chemical calculations[29−33] and the two-layer ONIOM (ONIOM2) method were used to study the nature of the intermolecular interactions between the water molecule and amino acid residues in the HIV-1 RT active site. Our aim is to gain an insight into the key role of the water molecule, which is essential for the HIV-1 RT active site. This work determined the water−amino acid residues interactions in order to understand the water orientation and interaction energy of the water molecule in the active site of HIV-1 RT.

2. COMPUTATIONAL METHODS

2.1. Structural Active Site Model. The geometry of HIV-1 RT and a water complex was obtained from the Protein Data Bank (PDB) entry code: 1VRT.[34] For the model system of the HIV-1 RT active site, four amino acid residues from p66 subunit were considered,[35] as shown in Figure 1.

These amino acid residues consist of Tyrosine183 (Tyr183), Methionine184 (Met184), Aspartic acid185 (Asp185), and Aspartic acid186 (Asp186). They are included in the Ω-loop of the active area of HIV-1 RT.[35] In this HIV-1 RT structure, there is only one water molecule which lies in the active site and the distance from an oxygen atom of Asp186 is 2.94 Å. Hydrogen atoms were used as the terminated atoms to saturate the model system of amino acid residues in neutral form.

2.2. Configurations of the Water Molecule. Based on X-ray crystallographic data, there is no hydrogen position information; therefore, numerous configurations of the water molecule in the active site were generated. The interaction between the water molecule and the Asp186 was first investigated (Figure 1), based on single point calculations at the HF/3-21G level. All calculations were carried out using the Gaussian 98 program[36] implemented in IBM/SP2 cluster. Starting configuration of water is kept constant and defined as water gas-phase geometry in which O−H = 0.96 Å and ∠H−O−H = 104.5°. The angles ∠O3−O4−C5 (A1) and ∠H2−O3−O4 (A2) and the dihedral angles O3−O4−C5−C6 (D1), H2−O3−O4−O5 (D2), and H1−O3−H2−O4 (D3) were optimized in the ranges $0° \le A \le 360°$ and $0° \le D \le 360°$ by the rotational step $\Delta D = 15°$. The translation of water molecule from the Asp186 was moved by a vector pointing from O4 to O3 within the range of O3−O4 distance (L); $1 \text{ Å} \le L \le 5$ Å. To get closer to the accurate configuration, translation steps with 0.5 Å were done, and additional steps with 0.1 Å between the optimum range were also reoptimized in order to search for a more precise configuration. Moreover, numerous configurations of the water molecule and the oxygen atoms of Tyr183 (atom O15) and the peptide bond between Tyr183 and Met184 (atom O16) were also considered and calculated in the same manner. Consequently, the optimal configuration of the water molecule in the active site was obtained. The binding energy (ΔE) of the active site and the water complex structure were calculated at HF/3-21G level with basis set superposition error (BSSE) corrections.

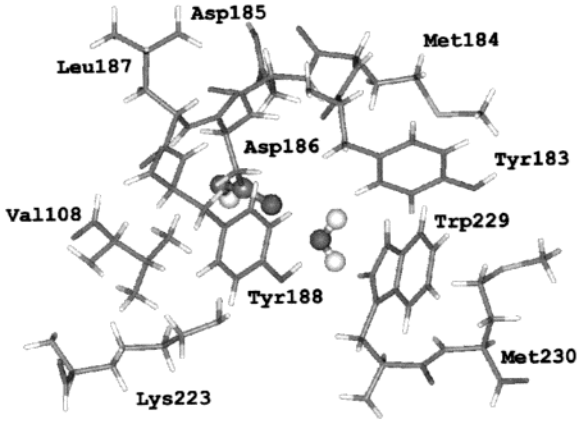

Figure 2. The model system for the ONIOM2 method; the inner layer is displayed in bond and stick and the outer layer is displayed in stick.

2.3. ONIOM2 Calculations. To investigate the interaction between the water molecule and the active site of HIV-1 RT, the effect of amino acid residues surrounding the water molecule in the active site were taken into account. Therefore, Asp186, Asp185, Met184, Tyr183, Leu187, Tyr188, Trp229, and Met230 were added to the model system consecutively. These amino acid residues were located within a 7 Å diameter from oxygen atom of the water molecule. In the present ONIOM2 calculations, the inner layer consists of part of Asp186 and the water molecule (Figure 2), which were treated with the higher level calculations. The remaining amino acid residues surrounding the water molecule (outer layer) were considered as the more realistic system and calculated at the lower level.

For the ONIOM2 calculations, the MP2/6-311G**, HF/6-31G**, HF/6-31G*, and HF/3-21G level of theory were applied. Consequently, the abbreviation of the combined methods are defined as the following: ONIOM2M = (MP2/6-311G**:HF/6-31G*)//(HF/6-31G**:HF/3-21G) and ONIOM2H = (HF/6-31G**:HF/3-21G). The orientation of water molecule in the inner layer was fully optimized, while those of other heteroatoms in the model system were kept

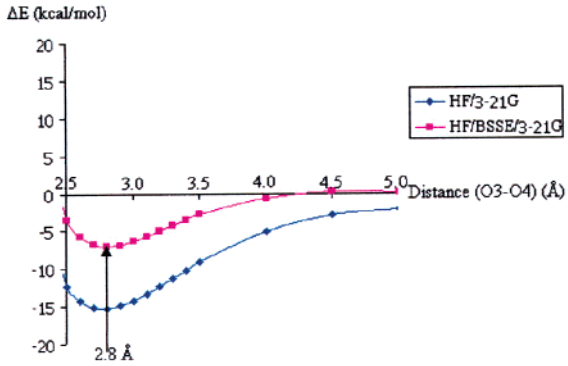

Figure 3. The relation between the most stable configurations, varying O3−O4 distances and the interaction energies (D2 = 175.4° and D3 = −0.6°).

frozen. The extrapolated energy for the ONIOM2 approach is defined as shown in eq 1

$$E(\text{ONIOM2}) = E(\text{High, Model}) + E(\text{Low, Real}) - E(\text{Low, Model}) \quad (1)$$

where High and Low refer to the high and low levels of calculations, respectively, while Model and Real refer to the small model and the real system, respectively, and *E*(High, Model), *E*(Low, Real), and *E*(Low, Model) are the total energies computed on the model system with the higher level of calculation and on both the real and model systems with the lower level of calculation, respectively. Furthermore, the full geometrical optimization of the water molecule at the HF/6-31G** level of theory was also performed.

The binding energy (ΔE_{ONIOM}) of the complex system can be obtained from eq 2

$$\Delta E_{\text{ONIOM}} = E_{\text{complex}} - (E_{\text{water}} + E_{\text{active site}}) \quad (2)$$

where energies of the system, E_{complex}, E_{water}, and $E_{\text{active site}}$, are ONIOM extrapolated energies of the complex, water, and active site, respectively.

3. RESULTS AND DISCUSSION

3.1. Orientation of the Water Molecule in the Active Site. To examine the orientation of the water molecule interacted with Asp186 in the active site, the angles A1 and A2 and the dihedral angles D1, D2, and D3 were generated, and single point calculations were done at the HF/3-21G level. It was found that the optimal configurations A1, A2, D1, D2, and D3 are 132.9 Å, 102.0 Å, 24.8°, 175.4°, and −0.6°, respectively. To find possible movement of the water molecule in the active site, the binding energies of complex structure were calculated at the HF/3-21G level with BSSE correction.

The obtained results as shown in Figure 3 indicated that the optimal distance between the oxygen atom of the water molecule (O3) and the oxygen atom of Asp186 (O4) is 2.80 Å. This optimal O3−O4 distance is shorter than that observed from X-ray crystallographic data of 0.14 Å. The binding energy of the complex structure was calculated and found to be −6.97 kcal/mol. The schematic representation of the possible movement and orientation of the water molecule in the active site is shown in Figure 4.

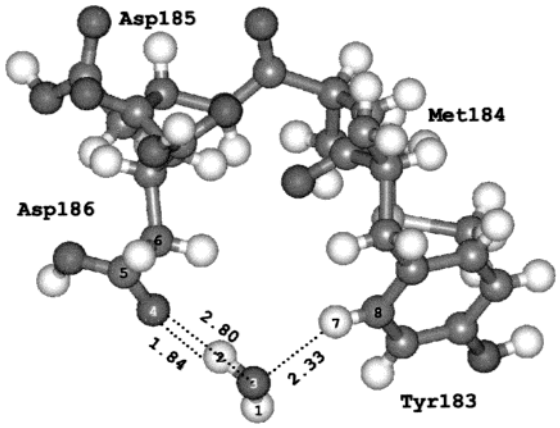

Figure 4. The optimal orientation of the water molecule, interacting with Asp186. The optimal orientation: L (O3−O4) = 2.80 Å, D2 (<H1 O3 O4 C5) = 175.4°, and D3 (< H2 O3 H1 O4) = −0.6°.

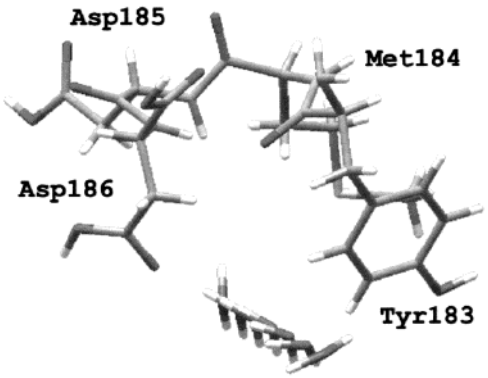

Figure 5. The movement of the water molecule to Asp186 in the active site of HIV-1 RT, based on HF/3-21G calculations.

Furthermore, the optimal orientations and the interaction energies between the water molecule and the oxygen atoms of Tyr183 and the peptide bond between Tyr183 and Met184 were also analyzed. Based on the obtained results, the binding energies of the optimal configuration of the water molecule interacting with the oxygen atoms of Asp186 and Tyr183 and the peptide bond between Tyr183 and Met184 are summarized in Table 1.

The derived binding energies indicated that the most preferable interaction between the water molecule and Asp186 is found. The moderate H-bonding between the water molecule and Asp186 is also possibly concerned as the distance between H2−O4 = 1.84 Å (Figure 4) and ∠O3−H2···O4, is 176.0°.[37] Moreover, the H-bonding between the water molecule and the hydrogen atom of the aromatic ring of Tyr183 also occurs as the distance H7−O3 = 2.33 Å and the angle ∠O3···H7−C8 = 156.6°. The obtained results provided information of the position and the orientation of the water molecule with respect to Asp186, as shown in Figure 5, and suggested that the water molecule can be stabilized by forming a hydrogen bond with Asp186.

3.2. Effect of Amino Acid Residues on the Interaction Energy. Additional amino acid residues, located in the active site within a 7 Å diameter from the oxygen atom of the water molecule, were considered and included in the model system consecutively (Table 2, models 1−8). ONIOM2M calculations were performed, and the results of the estimated binding energies between the water molecule and amino acid residues surrounding the water molecule and the relative stabilized

Table 1. Summary of the Optimal Configuration of the Water Molecule in the Active Site and the Binding Energies of the Water Molecule Interacting with Different Amino Acid Residues[a]

amino acid residues, interacting with the water molecule (O3)	optimal configuration (deg)						estimated binding energy (kcal/mol)
	A1	A2	D1	D2	D3	*L* (Å)	
Asp186 (O4)	132.9	102.0	24.8	175.4	−0.6	2.8	−6.97
Tyr183 (O15)	43.3	1.0	−178.8	−102.3	−2.0	6.0	14.76
peptide bond between Tyr183 and Met184 (O16)	128.7	1.0	136.9	−128.4	130.0	5.5	−2.56

[a] The calculation was performed at the HF/3-21G level with BSSE correction.

Table 2. Binding Energies between the Water Molecule and Amino Acid Residues in the Active Site within 7 Å, Calculated by the ONIOM2 Method

model	systems[a]	relative binding energy (kcal/mol)	
		ONIOM2M[b](ΔΔE)[d]	ONIOM2H[c](ΔΔE)
1	H_2O + Asp186	−7.7 (0.0)	−11.9 (0.0)
2	Model 1 + Asp185	−10.4 (−2.7)	−16.9 (−5.0)
3	Model 2 + Met184	−12.5 (−4.8)	−25.1 (−13.2)
4	Model 3 + Tyr183	−6.7 (1.0)	−13.4 (−1.5)
5	Model 4 + Leu187	−6.8 (0.9)	−13.5 (−1.6)
6	Model 5 + Tyr188	−15.8 (−8.1)	−26.2 (−14.3)
7	Model 6 + Trp229	−15.9 (−8.2)	−26.8 (−14.9)
8	Model 7 + Met230	−15.8 (−8.1)	−26.6 (−14.7)

[a] H_2O and acetic part of Asp186 were treated at the higher level and the other amino acids were added consecutively, based on the model 1. [b] ONIOM2M = (MP2/6-311G**:HF/6-31G*//HF/6-31G**:HF/3-21G). [c] ONIOM2H = (HF/6-31G**:HF/3-21G). [d] ΔΔE = stabilized estimated binding energy as relative to model 1.

energies (ΔΔ*E*) are presented in Table 2. For each calculation, the water molecule and part of Asp186 were treated at a higher level of calculations, and the rest of the system was calculated by the lower level.

As expected, the obtained results from ONIOM2M (MP2/6-311G**:HF/6-31G*)//(HF/6-31G**:HF/3-21G) and ONIOM2H (HF/6-31G**:HF/3-21G) calculations indicated that increasing amino acid residues can give higher binding energies. The increase of amino acid residues for up to six amino acids, Asp186, Asp185, Met184, Tyr183, Leu187, and Tyr188 (model 6), resulted in the consistency of the estimated binding energy reaching about −15.8 kcal/mol and −26.2 kcal/mol by ONIOM2M and ONIOM2H, respectively. The results indicate that inclusion of the dispersion interaction by using the MP2 level can bring more reliable results; on the other hand, the Hartree Fock level shows the overestimated binding energy. It can be noted that six amino acid residues surrounding the water molecule are sufficient to explain the environment enclosing the water molecule in the active site. Therefore, model 6 was selected to be used as a further model system to investigate the orientation of the water molecule in the active site.

Considering the obtained binding energies, models 1−3, addition of Asp186, Asp185, and Met184 surrounding the water molecule increased the binding energies from −7.7 to −12.5 kcal/mol. The reason for this is that in model 1, H_2O and Asp186 (Figure 6), three hydrogen bonds, O4···H2−O3, O3···H9−C6, and O3···H10−N11, can be observed, based on ONIOM2 calculations.

In model 2, the addition of Asp185 can lead to higher binding energy, compared to model 1. This can be explained by the fact that there are four hydrogen bonds, O4···H2−O3, O3···H9−C6, O3···H10−N11, and N12···H1−O3, occurring as shown in Figure 7.

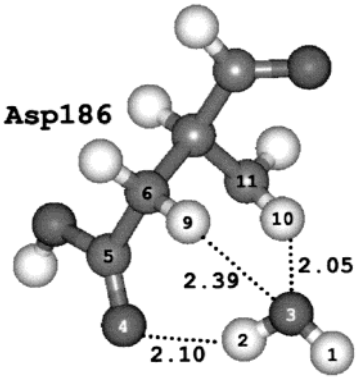

Figure 6. The water molecule in three H-bonding with Asp186 (model 1).

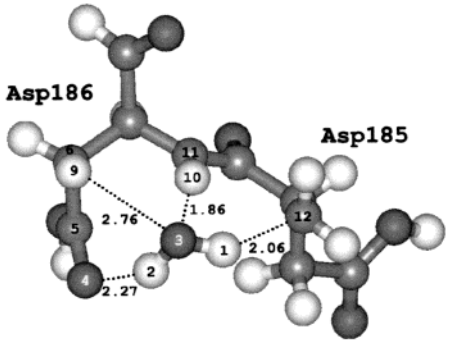

Figure 7. The water molecule in four H-bonding with Asp186 and Asp185 (model 2).

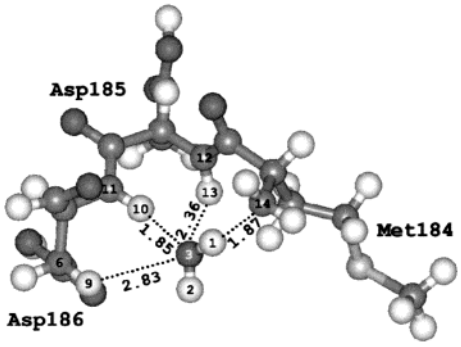

Figure 8. The water molecule in four H-bonding with Asp186, Asp185, and Met184 (model 3), introducing the water molecule in a cavity of the amino acids.

With addition of Met184 to model 2, resulting in model 3, the water molecule can form four hydrogen bonds, O3···H9−C6, O3···H10−N11, O3···H13−N12, and N14···H1−O3, as indicated in Figure 8, which introduces a cavity in the amino acids and a more stable complex structure.

However, addition of Tyr183 into the system (model 4) decreased the binding energy. This might be caused by the

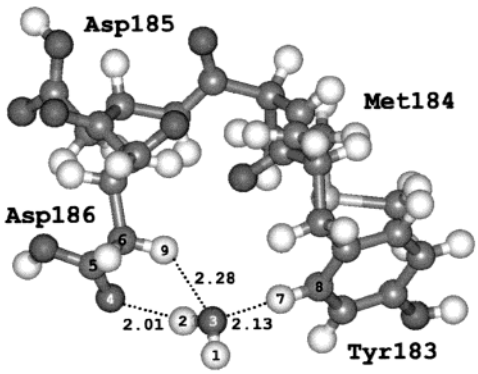

Figure 9. The water molecule in three H-bonding with Asp186 and Tyr183 (model 4) with lower binding energy caused by steric hindrance with Tyr183.

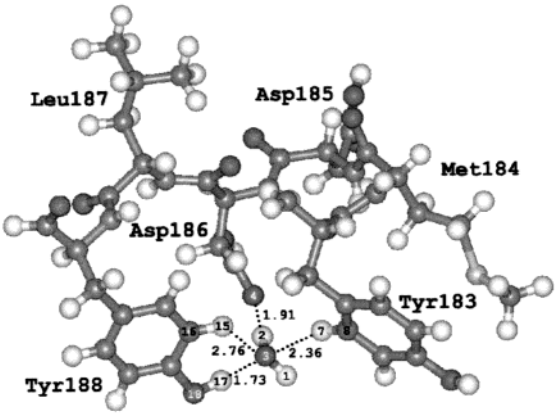

Figure 10. The water molecule in four H-bonding with Asp186, Tyr183, and Tyr188 (model 6), introducing consistent binding energy.

repulsion of the water molecule from the cavity of the active site due to the steric hindrance between the water molecule and Tyr183. In this model system, it is observed that the water molecule produced three hydrogen bonds with Asp186 and Tyr183, O4···H2−O3, O3···H9−C6, and O3···H7−C8, as depicted in Figure 9.

The inclusion of Leu187 into model 5 does not affect the binding energy of the system because this amino acid is located far from the water molecule. Nevertheless, in model 6, addition of Tyr188 is surprisingly helpful to give the most appropriate binding energy. The structural analysis indicated that there are four hydrogen bonds forming in this model, O4···H2−O3, O3···H7−C8, O3···H15−C16, and O3···H17−O18, as demonstrated in Figure 10. The explanation for this observation is due to stronger H-bonding existing in this system than those found in other models.

3.3. Orientation of the Water Molecule, based on ONIOM2 Calculations. Based on model 6, the orientation of the water molecule was investigated. It can be observed that the distances between the oxygen atom (O3) of the water molecule and the oxygen atom of Asp186 (O4), O3−O4, and the terminal oxygen atom of Tyr188 (O18), O3−O18, are 2.86 and 2.70 Å (Figure 10), which are shorter than the distances obtained from X-ray crystallographic structure of about 0.08 and 0.11 Å, respectively. The consistency of the binding energies of the complex system was also observed, based on models 6−8, and approximately found to be −15.8 kcal/mol or −8.1 kcal/mol, more stabilized as compared to the smallest complex model (model 1). It can be concluded

Table 3. Observed H-Bonding in the Model System, Obtained from Calculation by HF/3-21G, ONIOM2M (MP2/6-311G**:HF/6-311G*//HF/6-31G**:HF/3-21G) and Full Optimization at the HF/6-31G** Level of Theory

parameters	HF/3-21G	ONIOM2M	full optimization
R(H2−O4)	1.84	1.91	1.91
R(H7−O3)	2.33	2.36	2.44
R(H15−O3)		2.76	3.04
R(H17−O3)		1.73	1.85
A(O3−H2−O4)	176.0	176.3	175.2
A(C8−H7−O3)	156.6	161.3	157.7
A(C16−H15−O3)	-	121.4	120.9
A(O18−H17−O3)	-	174.4	170.9

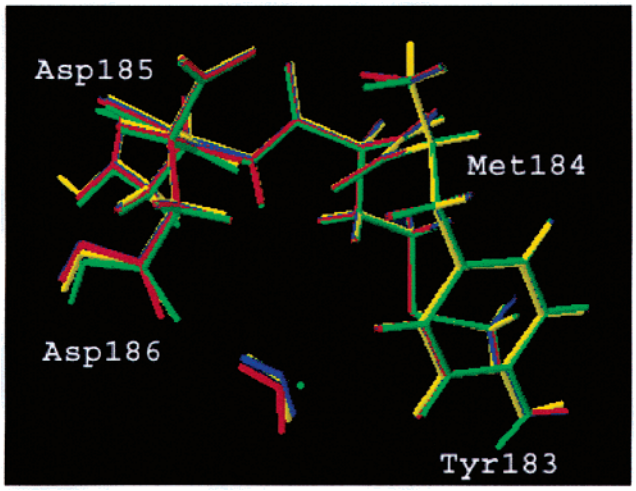

Figure 11. Superposition of the active site and the water molecule complexes, neglecting Leu187 and Tyr188: the structure from X-ray crystallography presented in green, the scanning (HF/3-21G) presented in yellow, ONIOM2M (MP2/6-311G**:HF/6-31G*//HF/6-31G**:HF/3-21G) method (model 6) presented in blue and full optimization (HF/6-31G**) presented in red.

that the water molecule plays a base−acid role, being hydrogen bond acceptor and hydrogen bond donor within the cavity.

Additionally, the results from the full optimization of the water molecule in the active site with six amino acid residues (model 6) were performed at the HF/6-31G** level of theory. The observed hydrogen bonds, obtained from different methods, HF/3-21G, ONIOM2M (MP2/6-311G**:HF/6-31G*//HF/6-31G**:HF/3-21G), and the full optimization (HF/6-31G**) are presented in Table 3, and it indicates good agreement between the results obtained from ONIOM2M and the full optimization. The superimposition of the complex structures is shown in Figure 11.

It was found that the results obtained by single point calculations scanning at the HF/3-21G level with BSSE correction and ONIOM2 method using model 6, agree well with the results from the full optimization of the water molecule at the HF/6-31G** level of theory. This can be denoted that the ONIOM2 method can lead to appropriate results in both the binding energy and the observed interaction of the water molecule in the active site of this model system.

4. DISCUSSION

Our theoretical results imply that the existence of the water molecule in HIV-1 RT polymerase active site plays an

important role in geometrical inducing of the surrounding amino acid residues. It is found from this study that the water molecule interacts by moderate H-bonding to Asp186 and Tyr183. In the polymerase domain, three carboxylate residues, Asp110, Asp185, and Asp186,[2] were found to be essential for the polymerase reaction. During binding of an incoming deoxyribonucleoside triphosphate (dNTP) and prior to nucleotide incorporation, RT must select the complementary base. Apart from the constraints imposed by base pairing, the structure of the polymerase active site is also supposed to be involved in the selection of the proper base. There is evidence that next to the three aspartate residues, several other less essential amino acids are in positions where they could affect the topology of the dNTP-binding site.[38] This can be supported by the results in Figure 10 as there are four H-bonding existing with Asp186, Tyr188, and Tyr183. Moreover, Met184 is the X-residue in the catalytically important TyrXAspAsp-motif found in all reverse transcriptases; it is in close proximity to the 3′-OH primer terminus and bound dNTP.[39,40] The geometrical inducing of amino acid residues obtained from this study seems likely to confirm the specific orientation of amino acid residues close to the polymerase active site.

5. CONCLUSIONS

The interaction between the water molecule and amino acid residues in the active site of HIV-1 RT was investigated using HF/3-21G with BSSE corrections and the ONIOM2M (MP2/6-311G**:HF/6-31G*//HF/6-31G**:HF/3-21G) and ONIOM2H (HF/6-31G**:HF/3-21G) methods. The HF/3-21G energies indicated that the derived binding energies of the water molecule and amino acid residues in the active site reveal the most preferable interaction between the water molecule and Asp186. Based on these results, the optimal orientation of the water molecule to Asp186 in the active site was observed. Additionally, the water molecule appreciated to form a moderate H-bond with Asp186 and Tyr183. The results from ONIOM2 calculations indicated that six amino acid residues, including Asp186, Asp185, Met184, Tyr183, Leu187, and Tyr188, surrounding the water molecule are sufficient to explain the environment enclosing the water molecule in the more realistic HIV-1 RT active site. Furthermore, four H-bonding was formed, and the binding energy of the complex system, based on ONIOM2 calculations, was found to be −15.8 kcal/mol, and the stabilized energy relative to the smallest complex model was −8.1 kcal/mol. It means that the water molecule presents the hydrogen bond donor and the hydrogen bond acceptor within the cavity. Interestingly, the orientation of the water molecule from the full optimization of the water molecule at HF/6-31G** level of theory confirmed the results obtained from the ONIOM2 calculations. These obtained results persuade us that the water molecule plays an important role in the active site of HIV-1 RT. The H-bonding is a rather strong interaction; thus, the water might induce the conformation of the active site to fit the catalysis process and helpfully attract dNTP to elongate the viral DNA in the replication process of this enzyme.

ACKNOWLEDGMENT

The authors thank Prof. Keiji Morokuma and Prof. Peter Wolschann for their valuable suggestions. Supa Hannongbua is grateful to the Thailand Research Fund and KURDI for research scholars (RSA440008). The RGJ-PhD program (3.C.KU/43/B.1), the Thesis and Dissertation Support Fund, Graduate School, Kasetsart University, and the Postgraduate Education and Research Programs in Petroleum and Petrochemical Technology (ADB-MUA) are gratefully acknowledged for scholarships and research supporting. The computing center, University of Vienna, the high performance-computing center of the National Electronics and Computer Technology of Thailand (NECTEC), the computer center of Kasetsart University, and Laboratory for Computational and Applied Chemistry (LCAC), Kasetsart University are also gratefully acknowledged for softwares and research facilities support. Thanks are also due to Anthony Reardon for reading of the manuscript.

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CI0203850