ภาคผนวก

Energetic Analysis for Y181C Mutant HIV-1 RT/Nevirapine Complex Using DFT, MP2 and ONIOM Calculations

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ABSTRACT

Quantum chemical calculations and the ONIOM method have been used to investigate the interaction of nevirapine with the binding site of HIV-1 RT, in regard to both the wild type and Tyr181Cys mutant systems. The particular interaction for each system was calculated using the B3LYP and MP2 methods with a 6-31G(d,p) basis set. Research results indicated that there are more attractive interactions between nevirapine and surrounding residues for the wild type as compared to the Tyr181Cys mutant HIV-1 RT. Consequently, the three-layered ONIOM (MP2/6-31G(d,p):B3LYP/6-31G(d,p):PM3) method has been utilized in the study of the structure and the binding energy in accordance with the ONIOM-BSSE scheme for binding energy correction of nevirapine at the binding site of HIV-1 RT, both in the wild type and in the Y181C mutant type structures. The present results clearly indicate that the Y181C substitution is more electrostatically repulsive than the wild type RT. This results in a decreasing of the stabilization energy of nevirapine and its binding pocket. The details concerning all interactions in the binding site for both systems give valuable guidance in regard to the design of more potent inhibitors.

KEYWORDS: Efavirenz, HIV-1 RT, K103N mutation, ONIOM and binding pocket

INTRODUCTION

Since the identification of the human immunodeficiency virus type 1 (HIV-1) as the causative agent of acquired immunodeficiency syndrome (AIDS) several classes of HIV-1 inhibitors have been found to exhibit a high specificity towards HIV-1, as well as a relatively low toxicity in cell culture and in patients.[1] The reverse transcriptase (RT) inhibitor has been shown to be a highly selective inhibitor of HIV-1 and specifically targets viral reverse transcriptase. Therefore, the RT enzyme is an important and excellent target in the design of inhibitors. This is because it plays a key role in the early stages of HIV replication, as well as not being required for normal cell replication and being a major specific target for the chemotherapy of HIV infections.[2-4] Two types of RT inhibitors, nucleoside inhibitors (NRTIs) and non-nucleoside inhibitors (NNRTIs), are among the most widely used drugs for the treatment of AIDS. However, the efficacy of these highly selective and potent inhibitors is limited by the emergence of drug-resistant mutants under drug selection pressure, both in vitro and in vivo.[5] Therefore the development of HIV-1 drug-resistant strains is one of the major concerns in AIDS therapy.[6] The resistant mutations are located at the residues within the drug binding site which are located around a hydrophobic pocket of HIV-1 RT in the p66 subunit. The RT inhibitors show differential activity against the mutant strains containing different amino acid substitutions in the RT enzyme and this results in resistance to the particular inhibitor in the presence of which it was selected.[7] The most common HIV-1 RT mutations associated with resistance to RT inhibitors are the Leu100ILe, Lys103Asn (K103N), Val106Ala, Val108Ile, Tyr181Cys (Y181C), Tyr188His and Gly190Glu substitutions.[8-12] The Y181C mutant form of HIV-1 RT is frequently found in the presence of RT inhibitors, and is resistant to most RT inhibitors.[13-14]

Nevirapine, developed by Merluzzi et al.,[15-16] is the first generation of NNRTIs approved by the American Food and Drug Administration (FDA) for clinical use and for the therapy of AIDS in 1996. Nowadays X-ray structures of the complex HIV-1 RT/nevirapine for both wild-type and mutant type is available in the Protein Data Bank (RCSB PDB, http://www.rcsb.org). Therefore, it may be useful to investigate the nature of the particular interactions between the nevirapine inhibitor and each amino acid in the binding site of the HIV-1 RT for both wild and mutant types. The binding site of the nonnucleoside inhibitors are located on the palm subdomain of the HIV-RT polymerase domain, approximately 10 Å from the polymerase active site and cause displacement of the catalytic location.[17-23] Consequently, the understanding of this particular interaction will be helpful in designing new potent inhibitors, especially, those which would be active against mutant enzymes.

In theoretical investigations of enzyme inhibitor interactions, the number of atoms in the molecular system makes it impossible to study them with great accuracy. In this case, study is also limited by the computational effort required. Recently, the development of multilayer integration methods in computational chemistry has made investigation of such large molecular systems more feasible. [24] Now, the ONIOM (Our own N-layered Integrated molecular Orbital plus molecular Mechanics) method has been introduced and improved its efficiency for over the years.[25-28] In the ONIOM approach, a large molecular system can be partitioned into multilayer regions, which are investigated by means of different theoretical approaches. The most important part of the molecule forms the innermost layer described at the highest theoretical level. Subsequent layers are treated by using progressively computationally simpler lower-level approaches.

Recently, Zhang et al., has reported quantum chemical calculations for the binding interaction between nevirapine and HIV-1 RT in both the wild type and in Y181C mutant structures. This has been done through the employment of the MFCC method executed at the HF/3-21G and B3LYP/6-31G(d) levels and computed in terms of fixed geometries for the crystal structures of the HIV-1 RT/nevirapine complexes.[29-30] Additionally, our previous

study also investigated the binding interaction for the HIV-1 RT/inhibitor complex using the ONIOM method.[31-34] In the present study, *ab initio* quantum calculation was used to study the nature of the particular interactions between nevirapine and each residue in the binding pocket of the Y181C mutant HIV-1 RT system and was executed by means of the MP2 method. Therefore, the three-layered ONIOM (ONIOM3) method was applied in examining the binding energy for the HIV-1 RT binding pocket/nevirapine complex. The understanding of the binding energy analyses of these complexes allows many of the basic features of the interactions with the nevirapine inhibitor to be determined and comparisons made between the wild-type[32] and mutant type systems in regard to inhibitor-enzyme interactions. Such fundamental knowledge will contribute to better understanding of the mutant HIV-1 RT/inhibitor binding mechanism and can be used as structural information relevant to the design of more potent inhibitors.

MODEL AND METHOD OF CALCULATIONS

For this study, the preliminary geometrical structure of the HIV-1 RT binding pocket/nevirapine complex for the Tyr181Cys mutant type was obtained from the 3.0 Å resolved crystal structures as found in the Protein Data Bank (PDB) with the PDB entry code 1JLB.[14] The focus of interest for investigation of the interaction under investigation is well-defined. For the area contains the residues surrounding the non-nucleoside inhibitor binding pocket (NNIBP) exhibiting at least one atomic interaction with all of the other atoms comprising the 7 Å diameter nevirapine structure. Although they manifest no significant interactions, the residues were also included to preserve the backbone geometries of the X-ray structures located between the amino acids within the NNIBP chain. Based on this assumption, the systems investigated consisted of the nevirapine bound into a NNIBP with 22 residues generated. Therefore, the residues investigated are Pro95, Leu100-Lys101-Lys102-Lys103-Lys104-Ser105-Val106, Val179-Ile180-Cys181, Tyr188-Val189-Gly190, Phe227-Leu228-Trp229, Leu234-His235-Pro236, Tyr318 in the p66 domain, and Glu138 in the p51 domain of RT. Assumed to be in their neutral form, all residues had their termini at the N- and C-terminal ends of the cut residues (see Figure 1), respectively, provided that they were not connected to another residue in the selected model by a link with an acetyl group (CH_3CO–) and a methyl group (–$NHCH_3$). Hydrogen atoms were added to the geometrical structure to generate the complete structure of the model system and their positions were optimized by the semi-empirical PM3 method. This structure was used as the starting geometry for all calculations.

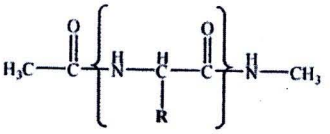


Figure 1. Capped groups of N- and C-terminal ends for amino acid chain.

The particular interaction energy (IE) between the nevirapine inhibitor and the individual residues, defined as X_i, was performed at the MP2/6-31G(d,p) level by using the starting geometries obtained from the X-ray diffraction structures based on the heavy atom fixing (HAF) optimization procedure, while the positions of hydrogen atoms were optimized using PM3 semi-empirical calculation. In order to evaluate the particular interaction, we also computed the basis set superposition error (BSSE) by means of an application of the counterpoise scheme of Boys–Bernardi[35] using the following Eq. (1).

$$IE^{CP}_{[NVP+X_i]} = E^{[NVP+X_i]}_{[NVP+X_i]} - E^{[NVP+X_i]}_{[NVP]} - E^{[NVP+X_i]}_{[X_i]} \qquad (1)$$

where $IE^{CP}_{[NVP+X_i]}$ is the counterpoise correction to interaction energy, $E^{[NVP+X_i]}_{[NVP+X_i]}$ is the energy of each nevirapine and residue pair complex within the basis set of each nevirapine and residue pair, while $E^{[NVP+X_i]}_{[NVP]}$ and $E^{[NVP+X_i]}_{[X_i]}$ are the energies of nevirapine and the individual residues, respectively, with the basis set of each nevirapine and residue pair.

In order to investigate the binding energy of nevirapine to the Y181C mutant HIV-1 RT binding site, the ONIOM3 (MP2/6-31G(d,p):B3LYP/6-31G(d,p):PM3) method was applied on the partitioned model system in three stages as shown in Fig. 2. The inner layer (region A) was treated at the MP2 method, the intermediate layer (region B) was treated at the B3LYP method, while the outer layer (region C) was treated at the PM3 method. The total ONIOM3 energy, E^{ONIOM3} of the entire system was derived from five independent energy calculations as shown in the following Eq. (2).

$$E^{ONIOM3} = E[MP2, A] + E[B3LYP, AB] + E[PM3, ABC] - E[B3LYP, A] - E[PM3, AB] \quad (2)$$

In the ONIOM3 optimization procedure, all atoms in the inner layer and in the intermediate layer (region AB) were relax, while the outer layer (region C) investigation was performed on the basis of HAF approximation. In order to analyze the binding energy based on the ONIOM results, we divided the ONIOM3 binding energy of nevirapine bound to HIV-1 RT, ΔE^{ONIOM3}, into three phases as shown in the following Eq. (3)

$$\begin{aligned}\Delta E^{ONIOM3} &= \Delta E(MP2, A) + [\Delta E(B3LYP, AB) - \Delta E(B3LYP, A)] + [\Delta E(PM3, ABC) - \Delta E(PM3, AB)] \\ &= \Delta E(MP2, A) + \Delta\Delta E(B3LYP, AB - A) + \Delta\Delta E(PM3, ABC - AB)\end{aligned} \quad (3)$$

This complex structure was evaluated by reference to the ONIOM-BSSE[31] corrected binding energy. The counterpoise correction (CP) was computed following Eq. (4). All calculations were carried out using the GAUSSIAN 03 package[36] running on Linux PC 3.2 GHz.

$$BE^{CP}_{ONIOM} = BE^{CP}_{MP2}[A] + BE^{CP}_{B3LYP}[AB] - BE^{CP}_{B3LYP}[A] \quad (4)$$

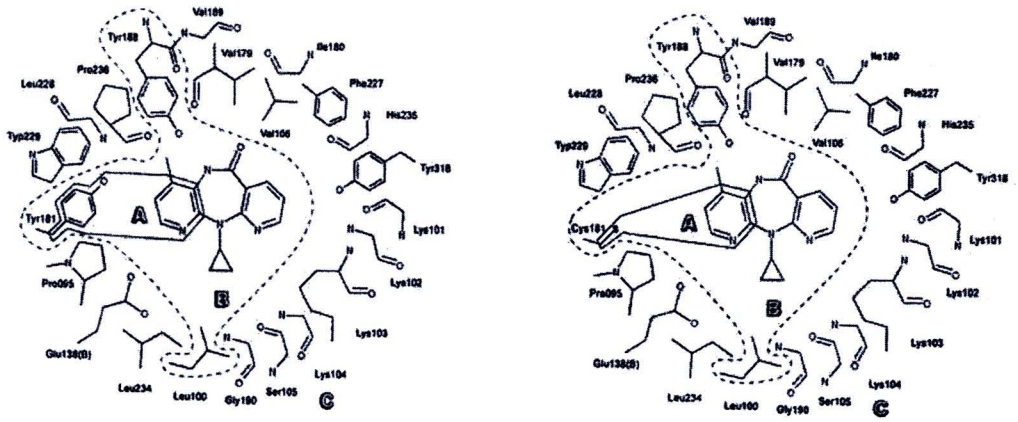


Figure 2. Adopted model system of nevirapine bound to the HIV-1 RT binding site. Layer partitioning is shown for the ONIOM3 model. (a) for the wild-type taken from ref. [32] and (b) for the Tyr181Cys mutant type.

RESULTS AND DISCUSSION

Interaction contribution of nevirapine with each residue in the binding pocket

The interaction energies between the nevirapine inhibitor and the individual residues, called generally X_i, in the binding site of wild type and Y181C mutant HIV-1 RT systems are given in Table 1. The interaction energy of each pair computed by B3LYP and MP2 methods with the 6-31G (d, p) basis set. Considering the outcome of calculations, it is clearly seen that there are more repulsive interactions between the nevirapine inhibitor and all residues surrounding the binding pocket for the Y181C mutant than is the case regarding the wild type. These repulsive interactions in the Y181C mutant are the origin of the reduced stability of nevirapine binding as compared to the wild type. Moreover, we found that the MP2 results produce more attractive interaction than the B3LYP method and the main interaction energies come from Leu100, Val106, Tyr181, Tyr188 and Trp229 for the wild-type and come from Leu100, Tyr188 and Trp229 for mutant type systems with interaction energies larger than 4 kcal/mol. The interaction energies of these residues with nevirapine are generally between 4-7 kcal/mol at MP2/6-31G(d,p) calculation. On the other hand, the interaction energies of Tyr188 and His235 are determined to be the main contributors to the wild-type and are not found to be main contributors in regard to the interaction between nevirapine and Tyr181 using the B3LYP method. In general, Tyr181 is the important residue in the binding pocket of HIV-1 RT because in its mutated form as Cys its sensitivity is significantly reduced.

Comparing the interaction energy between the wild type and the Tyr181Cys mutant HIV-1 RT systems, the attractive interaction between nevirapine and the residue at 181 is 5.81 kcal/mol for the former and is reduced to 3.40 for the latter using the MP2 method, which constitutes a decline of about 2.41 kcal/mol. Conversely, the B3LYP result exhibits no significant energy difference between the systems and the energy of the mutant system gives more attractive interaction than is the case with the wild type system. These results clearly show that the B3LYP method can not be used in exhibiting the intermolecular interaction between the H-atom and the aromatic ring of the amino acid. Therefore, the proper method to be used in studying the intermolecular interaction at the site of the aromatic ring should be investigated. Moreover, using the MP2 method, it was also determined that the interaction energies of Leu100 and Tyr188 clearly indicate stronger interaction than is the case with the other residues which are the main contributors for both the wild type and the mutant type systems. In addition, the attractive interaction between nevirapine and Try229 is almost conserved with the interaction energy being about 5 kcal/mol. Hence, these results are useful by virtue of providing the basis for ONIOM3 inner layer calculations for both wild type and mutant type structures which will be presented in the next section. Furthermore, the main focus of our study is to investigate binding energy differences between wild type and mutant type structures. Consequently, three amino acids, Leu100, Tyr181Cys and Tyr188, were investigated on the basis of considerations of the quantum level of calculation using the ONIOM3 method. Accordingly, these systems produce an identical ONIOM3 model which will be helpful in explaining the efficiency decrease in the operations of the nevirapine inhibitor against the Y181C mutant of HIV-1 RT.

Table 1 The interaction energies (kcal/mol) of nevirapine with individual residues (X_i), calculated by the B3LYP/6-31G (d, p) and the MP2/6-31G (d, p) methods.

Residues	Interaction energy (IE, kcal/mol)			
	Wild type		Y181C mutant	
	B3LYP	MP2	B3LYP	MP2
Pro095	2.36	1.15	-0.05	-0.73
Leu100	1.64	-3.09	1.98	-2.79
Lys101	-0.60	-1.15	-0.63	-1.06
Lys102	0.31	-0.31	0.33	-0.32
Lys103	0.61	-1.35	0.36	-1.37
Lys104	0.07	0.00	0.07	0.00
Ser105	-0.03	-0.10	-0.05	-0.15
Val106	0.97	-1.86	5.13	0.50
Val179	3.23	1.78	0.77	-0.44
Ile180	-0.01	-0.59	0.02	-0.41
Tyr181Cys	4.00	-2.77	1.94	-0.37
Tyr188	-0.53	-4.65	0.90	-5.04
Val189	0.19	-0.47	0.18	-0.69
Gly190	0.40	-0.41	0.50	-0.34
Phe227	-1.13	-1.88	-0.08	-1.71
Leu228	-0.18	-0.20	-0.31	-0.36
Trp229	0.51	-1.89	0.67	-2.88
Leu234	2.77	0.31	4.20	1.22
His235	-1.45	-2.24	-0.97	-1.66
Pro236	-0.24	-1.42	2.73	1.54
Tyr318	-0.09	-1.24	1.09	-1.44
Glu138(b)	0.06	-0.82	-0.51	-1.05

The structure and binding energy of the Tyr181Cys mutant of the HIV-1 RT's binding site/nevirapine complex

The main purpose of our study is to investigate the specific interaction of nevirapine with the binding site of HIV-1 RT both in its wild type and its Tyr181Cys mutant. However, this complex structure is too large for MP2 calculations. Therefore, the ONIOM method has proved to be a powerful method to study the biological systems. Furthermore, regarding our earlier study of the binding site of the HIV-1 RT/nevirapine complex using the ONIOM method,[31-32] results show that the interaction between the nevirapine inhibitor and the Tyr181 form H...π, as well as the 181Cys in the mutant structure, could be treated in a fashion such that computational complexity was reduced. The present study has focused on the specific interaction between nevirapine and the amino acids at the 181 position. The other significant amino acids were treated by means of the B3LYP method. Therefore, we divided the model system into three parts and applied the three-layer ONIOM3 method as already described and as shown in Figure 2. The binding energies and its components were corrected on the basis of the ONIOM-BSSE scheme.

Table 2 shows the binding energy and their components of nevirapine when bound to the binding site of HIV-1 RT in both the wild type and the Y181C mutant type systems. It was found that the binding energy for the Y181C mutant is -5.59 kcal/mol. When the superposition error was corrected using the counterpoise method based on the ONIOM-BSSE scheme, the binding energy was found to have been reduced to -4.65 kcal/mol. The absolute binding energy differences between the wild type and the Y181C mutant type are 9.24 and 4.24 kcal/mol, respectively, on the basis of ONIOM3 and ONIOM-BSSE scheme calculations. Furthermore, the total binding energy of nevirapine in the binding pocket of HIV-1 RT derived from ΔΔE (Low, ABC-AB) is -9.19 kcal/mol and -9.32 kcal/mol for the wild type and the Y181C mutant type, respectively. In analyzing the components of the ONIOM binding energies involving the interaction energy in the region A, ΔE(MP2, A), they are found to have an effect on the wild type and the Y181C mutant by decreasing the binding energy from -3.62 to -0.44 kcal/mol and from -1.43 to 1.31 kcal/mol, respectively, in accordance with the ONIOM and ONIOM-BSSE scheme. This consideration of the binding energy leads to another strong argument for explaining why nevirapine has higher biological activity in regard to the wild type as compared to the Y181C mutant enzyme. Consequently, these results clearly show that the Y181C mutation decreases the stabilization energy of nevirapine bound to its binding pocket when compared to the wild type. Moreover, the binding energy in the region B, ΔΔE(B3LYP, AB-A) is reduced from -2.02 to 3.86 kcal/mol on the basis of the ONIOM-BSSE scheme, while the binding energy in this region is concomitantly reduced from 1.83 to 3.36 kcal/mol. These effects imply that the relaxation of residues during optimization do not have as strong effects on the binding energy obtained on the basis of medium level and high level calculations.

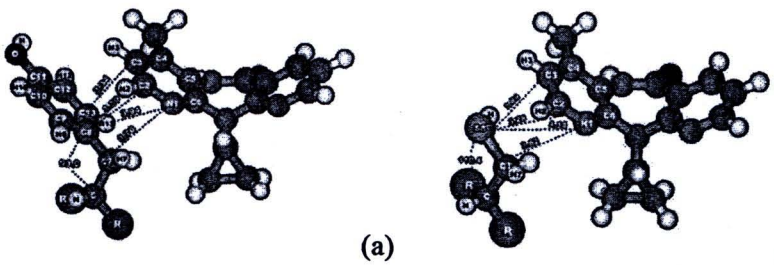
Considering the binding energy components, the results show that both in region A and in region B of the wild type an attractive interaction is generated. On the other hand, the Y181C mutants are found to have an attractive interaction only in region A, but yet produce a repulsive interaction in region B. Including the ONIOM-BSSE, only region A of the wild type system generates attractive interaction. Consequently, it may be concluded that the interaction between nevirapine and Tyr181 plays an important role in the binding pocket of the HIV-1 RT/nevirapine complex because the mutation to 181Cys significantly reduces its sensitivity. Thus, it can be seen that the MP2 method allows for specifying intermolecular interactions in the aromatic ring.

Figure 3 shows the selected optimized distances between heavy atoms in the interaction core region treated at the MP2 level of calculation between nevirapine and Tyr181 for the wild type and 181Cys for mutant systems computed at ONIOM3 as derived from the HAF

optimized procedure, as previously explained. The wild type results show the distances between N1-C7 and C2-C8 to be about 3.76 and 3.39 Å, respectively. As a result, we can see that the wild type yields longer interfragment distances than is the case for the mutant results for N1-C7 and C2-C8 as wild types or C2-S8 as mutant systems. The results can be easily explained in terms of the repulsive interfragment interaction between nevirapine and Tyr181. Therefore, the binding energy calculation in this region requires taking the dispersion interaction into account. Moreover, the H-atom of nevirapine is directed towards the inner region of the tyrosine ring. In addition, the nitrogen atom (N1) of nevirapine forms hydrogen bonding with the hydrogen atom of Tyr181 at C7 which then produces the facial H...π interaction. This finding is the main cause of the stronger interaction of the wild type as compared with the mutant system.

CONCLUSIONS

This work was performed in order to investigate the geometry and binding energy of the complex formed between nevirapine and the HIV-1 RT binding pockets both in the wild type and Tyt181Cys mutant structures. The ONIOM-BSSE scheme was performed on the ONIOM optimized geometry for binding energy correction. Investigation of inhibitor enzyme interactions for the nevirapine inhibitor against the wild type and Tyr181Cys mutant of HIV-1 RT binding pocket showed there are more attractive interactions between nevirapine and surrounding residues. However, the interaction energies of Leu100, Tyr181 or 181Cys and Ty188 clearly showed stronger interaction than the other residues which are the main contributors in both systems as determined by the MP2 method. The ONIOM results derived in this study show that the complex structure forms the hydrogen bonding between the nitrogen atom of the pyridine ring and the hydrogen atom of carbon beta at residue 181. The analysis of the ONIOM-BSSE energy shows that the binding energy in the interacting core region (small model) is very weak and the substantial binding energy comes almost exclusively from the interaction of nevirapine with other residues in the binding pocket. The three-layered ONIOM (MP2/6-31G(d,p):B3LYP/6-31g(d,p):PM3) calculations have been successfully applied to determine the binding energy of the nevirapine inhibitor bound to the HIV-1 RT binding pocket. In comparing the wild type and Y181C mutant type, results clearly indicate that the Y181C substitution is more electrostatically repulsive than the wild type RT which is affected with the consequence that there is a decrease of the stabilization energy of nevirapine bound to its binding pocket. Finally, this study shown that it is possible to apply the combined high and low quantum chemical methods based on various approaches such as Møller-Plesset perturbation theory, Density Functional Theory (DFT) and semi-empirical methods studying bimolecular systems in a fashion such that it is feasible to correct ONIOM energy by using the ONIOM-BSSE scheme. Finally, the three-layered ONIOM method is recommended as the best compromise when combined with other methods in future studies of similar inhibitor-enzyme interactions



(a) (b)

Figure 3. Optimized structure of the nevirapine and Tyr181 complex from ONIOM3 based on HAF with selected atomic labels, bond distances and bond angles; (a) for the wild-type and (b) for the Tyr181Cys mutant type.

Table 2. Binding energies (ΔE, kcal/mol) and their components for nevirapine and the HIV-1 RT complex for the wild type and Y181C mutant systems, optimized using the ONIOM3 (MP2/6-31G(d,p):B3LYP/6-31G(d,p):PM3) method; all ONIOM-BSSE corrected binding energy and their components are in parenthesis.

Energy Components	Wild type[a]	Y181C mutant type
	ONIOM	ONIOM
ΔE	-14.83 (-8.79)	-5.59(-4.65)
ΔE (High, A)	-3.62(-1.43)	-0.44(1.31)
ΔΔE (Mid, AB-A)	-2.02(1.83)	3.86(3.36)
ΔΔE (Low, ABC-AB)	-9.19	-9.32

[a] The interaction energy taken from Ref. [32].

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