al.[39] Characteristics of the simulated systems are summarized in Table I. The following partial relaxations were applied while keeping the remaining structure fixed: first the hydrogen atoms, the newly added residues, and then the solvent molecules. The energy of the whole system was minimized by 100 steps using the steepest descent method followed by another 9900 steps using the conjugate gradient method. The system was thermalized from initial 10 K to the temperature of 300 K over 50000 iterations (500 ps) and then equilibrated for 1 ns. The Particle Mesh Ewald (PME) method was used to calculate the full electrostatic interaction with 12 Å was cut off.

Insert Table 1

The MD simulations were carried out in an isobaric-isothermal ensemble (NPT) with a constant pressure of 1 atm and the temperature of 300 K. The external coupling bath[40] with coupling constant of 0.2 ps was employed in order to keep the pressure constant throughout the simulations. A 2-fs time step was applied with SHAKE to constraint all bonds involving hydrogen atoms. Snapshots of coordinates of enzyme structure and water molecules were collected every 1 ps along the 2 ns MD simulations yielding 2000 frames stored for the trajectory analyses. The trajectories of both systems, FULL+ION-INH and CORE-INH, were analyzed using the PTRAJ and CARNAL modules in AMBER7. Three dimensional structures of the enzyme were visualized using the programs Molmol,[41] Rasmol[42] and Swiss-PDB Viewer.[43] The quality of the protein structures were evaluated using the Procheck program[44].

3. Results and Discussion

3.1 Stabilities of the complexed systems

Thermodynamic properties, energies and temperature, were monitored to examine stability of the two systems, FULL+ION-INH and CORE-INH (Fig. 2). The profiles displayed in Figure 2 indicate reliability and stability of the two systems. Two systems show the same average temperatures value of about 300 K.

Insert Figure 2

3.2 Dynamical behavior of the complexed systems

The root-mean-square deviations (RMSD) of the C_α atoms with respect to the average structure over the 2-ns simulation time of the CORE-INH (Figure 3a) and FULL+ION-INH (Figure 3b) were evaluated. Throughout the simulation, the whole structure of FULL+ION-INH is, as expected, more flexible than the CORE-INH systems. However, considering only the core part, it can be shown that the RMSD of both systems are almost equal (Figure 3). Binding of the inhibitor causes the RMSD decrease up to 1 Å (Figure 3a). This differs from previous simulation[29] where we showed up that the presence of the two end terminal affect the structure to be less flexible. The RMSD per residue plot (Figure 4) displays the higher flexibility of the FULL+ION-INH system. The plot (Figure 4b) shows also that the two ends of both systems, with and without the ions, are highly flexible, especially the C-terminal, the DNA binding domain. The large fluctuation of the C-terminal is due to the long linker (~10 amino acid residues) between the core and the C-terminal domains. This is consistent with our previous study[29].

Insert Figure 3

Insert Figure 4

3.3 Conformation of the active site residues

The active site region on the core domain, a DD35E motif, contains two aspartic and one glutamic residues. The Mg^{2+} cation is bound to these residues. The coordination of the ion and two conserved aspartate residues as well as their torsional angle (χ_a) were defined in Figure 5.

Insert Figure 5

To understand the structure of the catalytic region, especially how the presence of the inhibitor affects the cavity for inhibitor binding of this region, distributions of the selected distances and torsional angles of the three systems (FULL+ION-INH and CORE-INH) over the simulation time were evaluated and plotted in Figures 6-8.

In terms of the cation's coordination, distributions of the distances between electron donor atoms of the catalytic residues and the Mg^{2+}, *d1-d4* shown in Figure 5, for the three systems were plotted in Figure 6. Comparisons with the available data, the cation's coordination from the previous studies[29,37] were also summarized in Table 2. It can be seen from Table 2 that the X-ray data, as well as the simulation results by Lin *et al.*[29,37] predict that the Mg^{2+} coordinates with 4 oxygen atoms from the water molecules and 2 carboxyl oxygen atoms of the Asp64 and Asp116. The experimental Mg^{2+}-O distances are in the range 2.10 – 2.91 Å while those of Lin *et al.* were not reported. For the Luca *et al.* dimer full-length structure where two Mg^{2+} ions per monomer were taken into account[29], the coordination number was not reported. However, with an assumption that binding could be formed when the Mg^{2+} - O distance is shorter than 3 Å, we measured all the Mg^{2+} - O distances in their modeled structure and found that the coordination number can be either 3 or 4.

From the distribution plot (Figure 6) and the optimal Mg^{2+} - O distances (maximum of the peak) (Table 2), our results for the CORE-INH and FULL+ION-INH also indicate that the coordination number for the Mg^{2+} is 6 but the specific binding structure is different from the previous studies.

For the CORE-INH system, the octahedral sites of our data of Mg^{2+} contain two oxygen atoms of Asp64, two oxygen atom of Asp116 and two oxygen atoms of the water molecules (Figure 6a). However, our solvation properties are, quantitatively, in the range obtained from the MD results of the full-length structure by Luca *et al.* which observed 1-4 bonds between the Mg^{2+} and the catalytic residues and the corresponding Mg^{2+} - O distances in the range of 1.75 – 2.31 Å. This is different from the free CORE system in which the three-coordination was found. For the FULL+ION-INH, the octahedral sites of our data of the Mg^{2+} contain two oxygen atoms of Asp64, one oxygen atom of Asp116 and three oxygen atoms of the water molecules. This is consistent with our previous data of the free form full-length (Figure 6b).

It can be seen from the maxima of the distribution plots for the four (*d1-d4*) and three (*d1-d3*) distances to the carboxyl oxygen in the CORE-INH and the FULL+ION-INH, respectively, (Figure 6 and Table 2) that significant changes of the Mg^{2+} - O distances were observed in comparison between the CORE-INH and FULL+ION-INH systems. One more oxygen atom from Asp116 coordinates with Mg^{2+}. This is different from our previous study on the free enzyme core only system while the full-length are still the same. Note that the distances of 3.53 Å for the FULL+ION-INH are shorter than the FULL+ION (3.89 Å) but still too far to coordinate to the Mg^{2+}.

Some comments should be made concerning the *d1* - *d4* in which the distances observed from our study for both CORE-INH and FULL+ION-INH systems are notably shorter than those of the other works. However, our Mg^{2+} - O distances ranging from 1.86 – 1.93 Å agree with those of Mg^{2+} in aqueous solution of 2.0 – 2.05 Å obtained from the simulation using *ab initio* fitted potentials[45] and the QM/MM simulations[46].

Insert Figure 6

Insert Table 2

In addition to the ion's coordination numbers and the related distances which represent the size of the catalytic site of the HIV-1 IN, binding affinity between the enzyme and the inhibitor was known to depend, obviously, on the orientation of the catalytic residues. Such information for the CORE-INH and FULL+ION-INH was evaluated and displayed in Figure 7 - 8 in terms of torsional angles as defined in Figure 5b and 5c. The plot indicates that no preferential conformation was found for the Glu152. Its conformation is highly flexible in all both complexed systems (Figure 7a and 8a). This is understandable because this residue is not located within the solvation shell of the Mg^{2+}. The detected data agree well with the previous report on the catalytic core domain by Lins *et al.* which stated that the Glu152 shows large fluctuations[35]. The results are similar to the free enzyme systems[29].

For the Asp116 catalytic residue, there found only one preferential conformation in the both systems. The result of the CORE-INH system where one stable conformation at 134.83° observed is similar to what reported in the free CORE at -165° in our previous MD result[29] (Figure 7b). The discrepancy observed for the conformation between the CORE-INH and CORE is likely due to the presence of the 5-CITEP inhibitor. In contrast, the full-length systems complexed with 5-CITEP shows only one preferential conformation at -112.86 while the most stable structure at -145° and another minor conformation at 155° were found in the free full-length (FULL+ION)[29] (Figure 8b).

For the Asp64, it is likely to have only one preferential conformation at the torsional angles -0.52 and 19.45 for the CORE-INH and FULL+ION-INH, respectively, see Figure 7c and 8c. These are comparable to the previous results where the one stable conformation at the torsional angles of 5° and 13° were observed for the CORE and FULL+ION systems, respectively[29]. The plot indicates clearly that the presence of the inhibitor in the active site leads to remarkable changes of the orientation of the catalytic residues, especially for the Asp64 and Asp116.

Insert Figure 7

Insert Figure 8

The dynamical behaviors of the missing region, residues 140 – 149, were investigated to explore the conformational change of this region. The results are directly compared with those of subunit A of Luca's simulation[37] and our previous simulation for the FULL+ION[29] (see Table 3). The investigated distances as well as the corresponding standard deviations obtained from our simulation were distributed in a narrower range, the differences between the maximum and the minimum distances, in comparison to those of Luca's[37] and our previous MD[29] works. However, the distances measured between the C_α atoms of the missing residues (140-149) to the Mg^{2+} of the complexed systems are much lower than that of the free form one (Table 3).

Insert Table 3

4. Conclusion

Molecular dynamics simulations were investigated for the two complexed systems between HIV-1 integrase (IN) and 5-CITEP for the core domain only (CORE-INH) and the full-length structure (FULL+ION-INH). This aimed to investigate the difference in the molecular properties of the full-length and the core only domain due to the effects of the two ends, C- and N-terminal, on those properties in the interaction with inhibitor in the core domain.

The full-length structure and the core only domain prepared by our group (FULL+ION and the CORE) were used as starting structure. The 5-CITEP inhibitor taken from the crystal structure of 1QS4 was substantially added to the starting structures by superimposition onto the core part. The simulations results obtained showed that the presence of the inhibitor in the active site decreases the mobility of the CORE system significantly as the RMSD for the CORE and the CORE-INH systems are 1.73 and ~1 Å, respectively. In contrast to the full-length systems, the pattern of the RMSDs plot for the free and complexed forms are likely similar.

The presence of the inhibitor in the active site was also found to affect the conformations of the catalytic triad. These are shown in term of the torsional angles. The differences were found between the free and the complexed forms. Only one preferential conformation was found for the Asp64 and Asp116 in both systems. Binding to the inhibitor induce the two preferential conformations of the Asp116 to form only one stable conformation for the full-length system. In addition, no preferential conformation was found for the Glu152 in both systems.

The differences between those of the core-only and the full-length structure confirm our previous MD results of the free enzyme that the full-length HIV-1 IN is needed for drug screening and drug design purpose.

Moreover, we are currently studying the structure and dynamics of the HIV-1 IN binding to DNA in the presence of metal ions and inhibitor in the active site to provide understanding on the interaction between the HIV-1 IN and DNA. Results of such a study will be reported in a forthcoming paper.

Acknowledgement

SP would like to acknowledge the Thailand Research Fund (TRF) grant number MRG4680201 for financial support. AW is gratefully to the Royal Golden Jubilee (RGJ) Ph.D. program and the Thailand Research Fund (TRF) for all funding. We thank for all computing facilities provided by the Computational Chemistry Unit Cell (CCUC) at Department of Chemistry, Faculty of Science, Chulalongkorn University.

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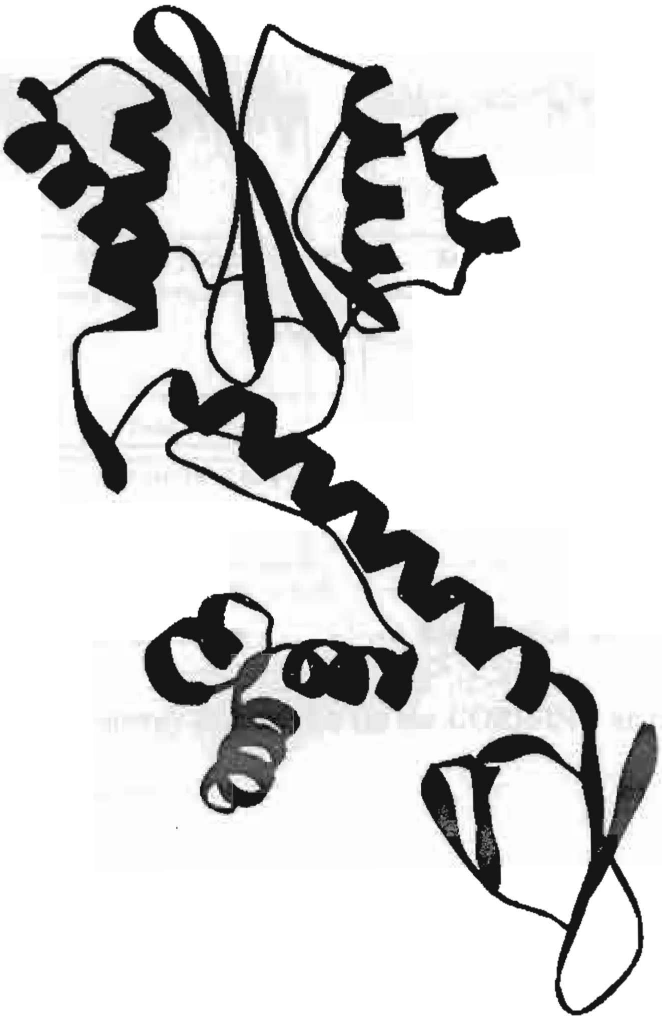


FIGURE 1 Full-length model of the HIV-1 IN[29].

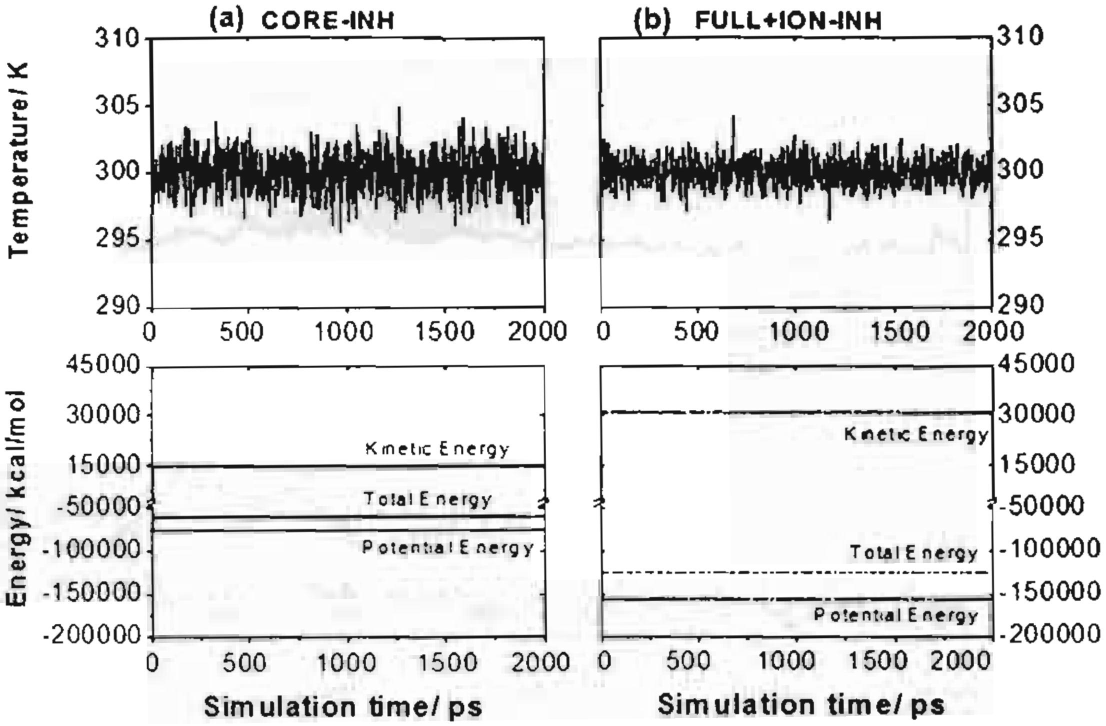


FIGURE 2 Temperature and energy profiles for (a) the CORE-INH and (b) the FULL+ION-INH over the 2-ns simulation time.

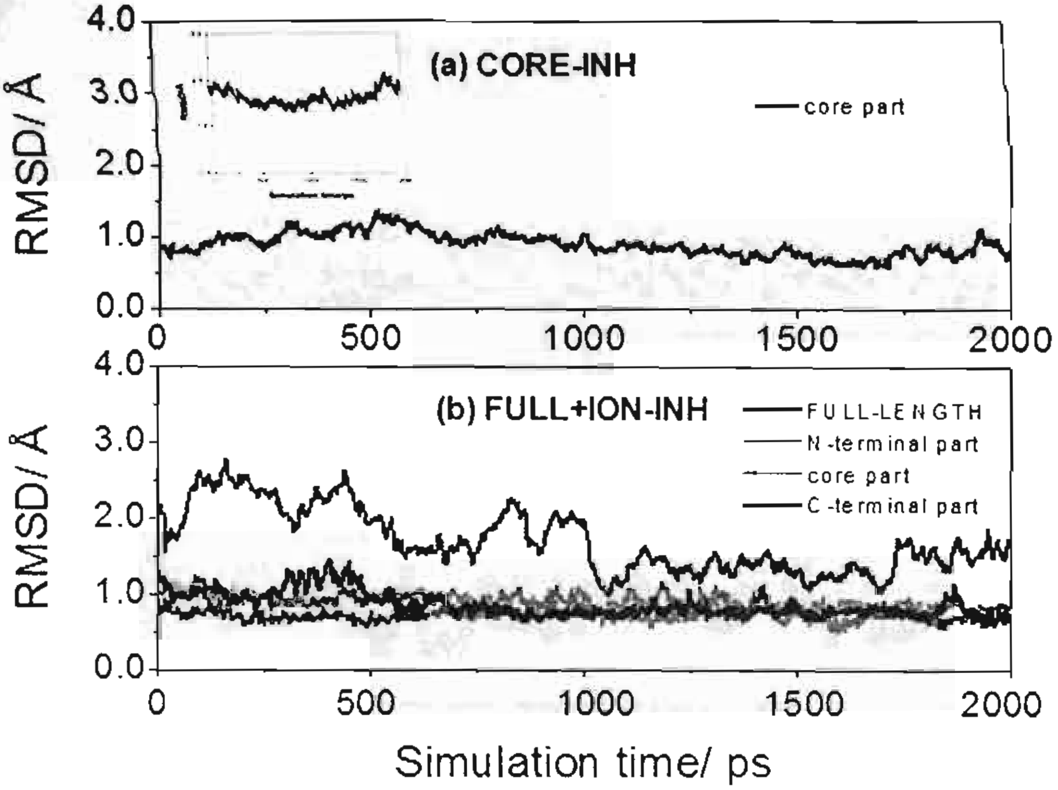


FIGURE 3 RMSD of the Cα of the HIV-1 IN with respect to the average structure as a function of the simulation time of (a) CORE-INH and (b) FULL+ION-INH. The RMSD of the free CORE system was given as an inset.

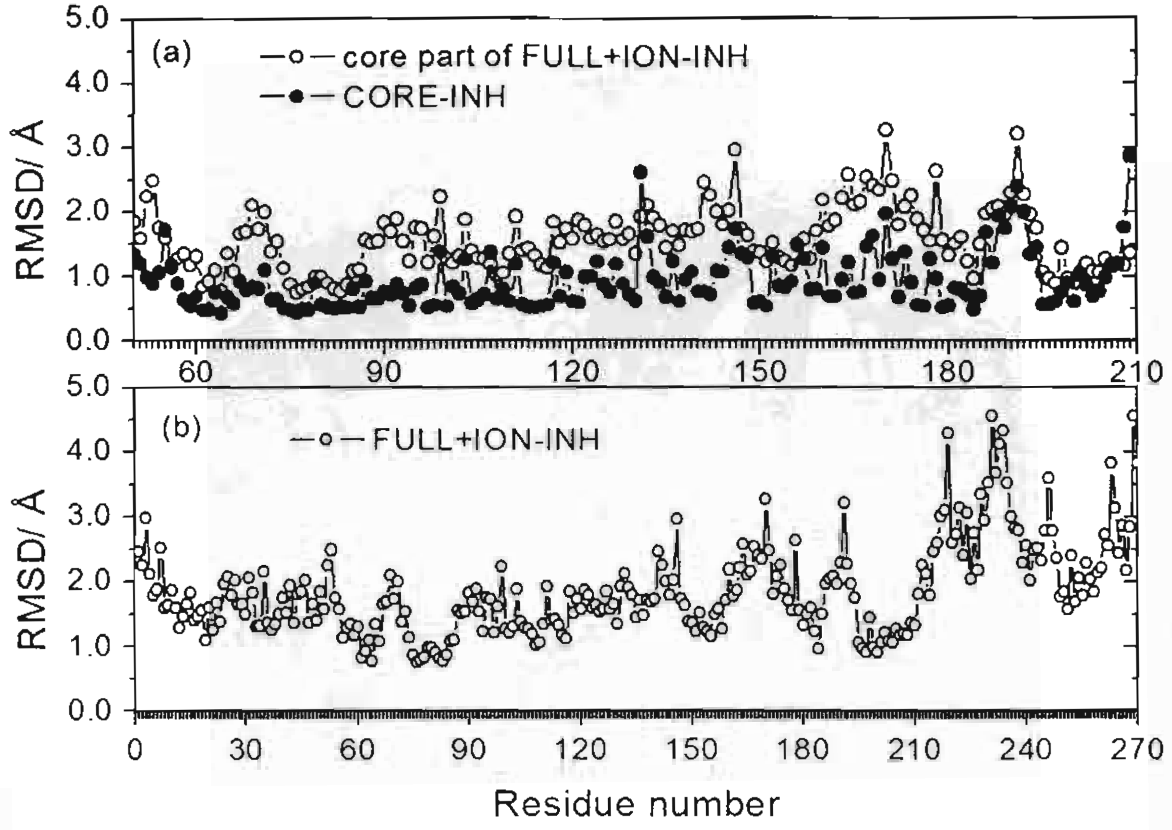
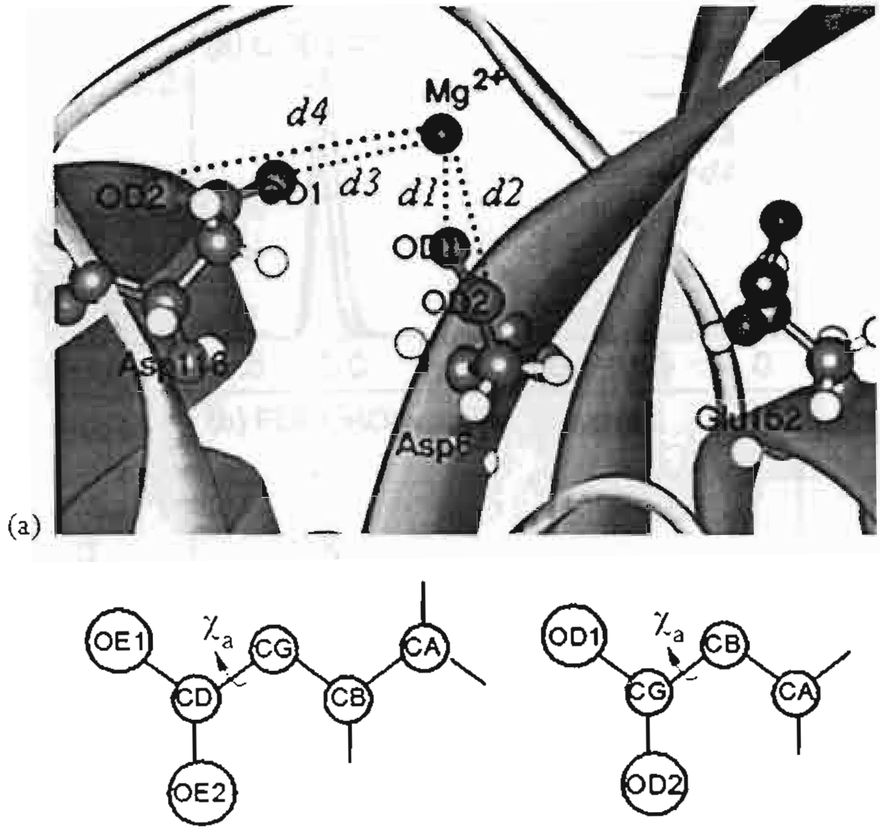


FIGURE 4 RMSDs of the Cα of the HIV-1 IN with respect to the average structure as a function of the simulation time plotted for (a) the core part of the FULL+ION-INH and CORE-INH systems, (b) the whole full-length of FULL+ION-INH, calculated by fitting onto its corresponding reference structure.



(b) Glutamic acid side chain

(c) Aspartic acid side chain

FIGURE 5 (a) Schematic representation of the catalytic region of the core domain of the HIV-1 IN consisting a Mg^{2+}, the two aspartate residues (Asp64 and Asp116) and a Glu152 for the two simulations, (b) side chain of glutamic acid and (c) side chain of aspartic acid.

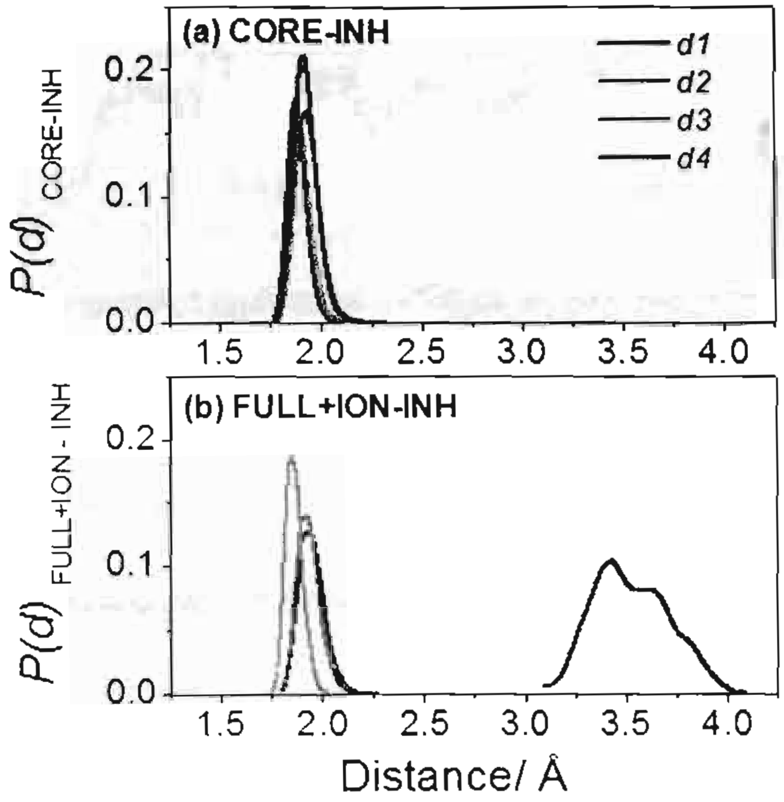


FIGURE 6 Distribution of the distance P(*d*) between Mg^{2+} and the carboxyl oxygen of the catalytic triad residues, *d1*, *d2*, *d3* and *d4*, as for Asp64:OD1, Asp64:OD2, Asp116:OD1, Asp116:OD2, respectively, for the CORE, FULL+ION and the FULL systems. The arrows illustrate the data extracted from the two subunits of Luca's structure.

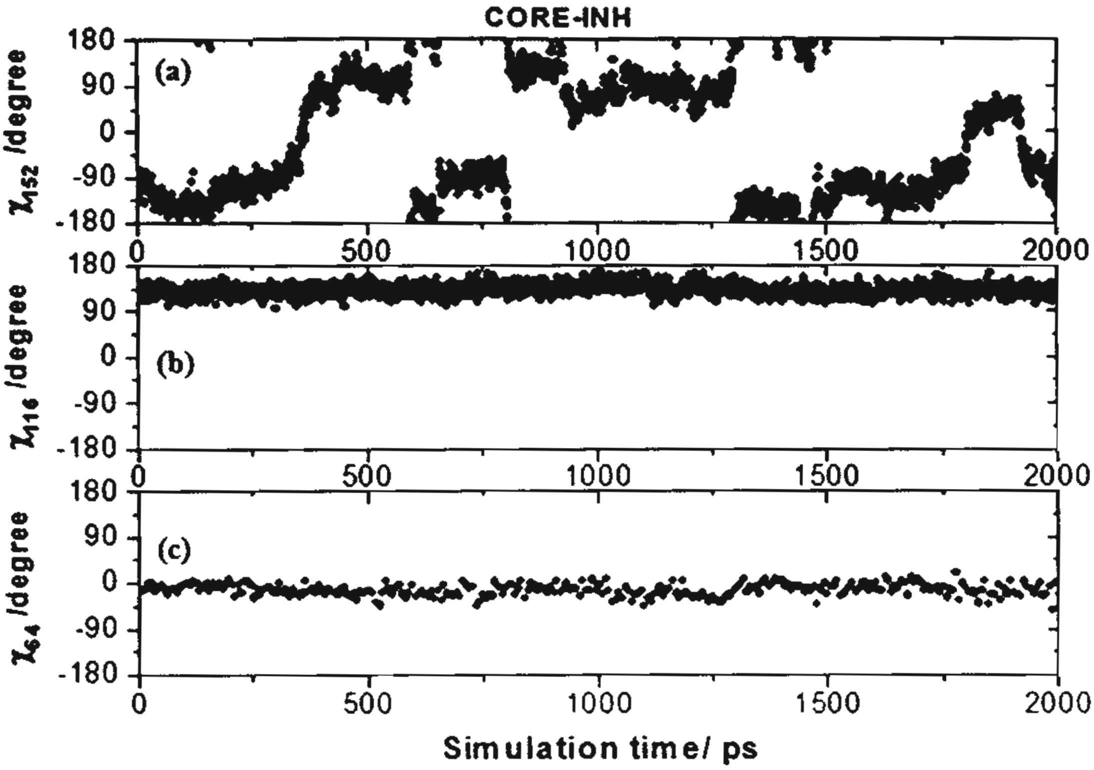


FIGURE 7 Torsional angles (χ_a) of the catalytic triad (a) χ_{152} (b) χ_{116} and (c) χ_{64} throughout the simulation of the CORE-INH system.

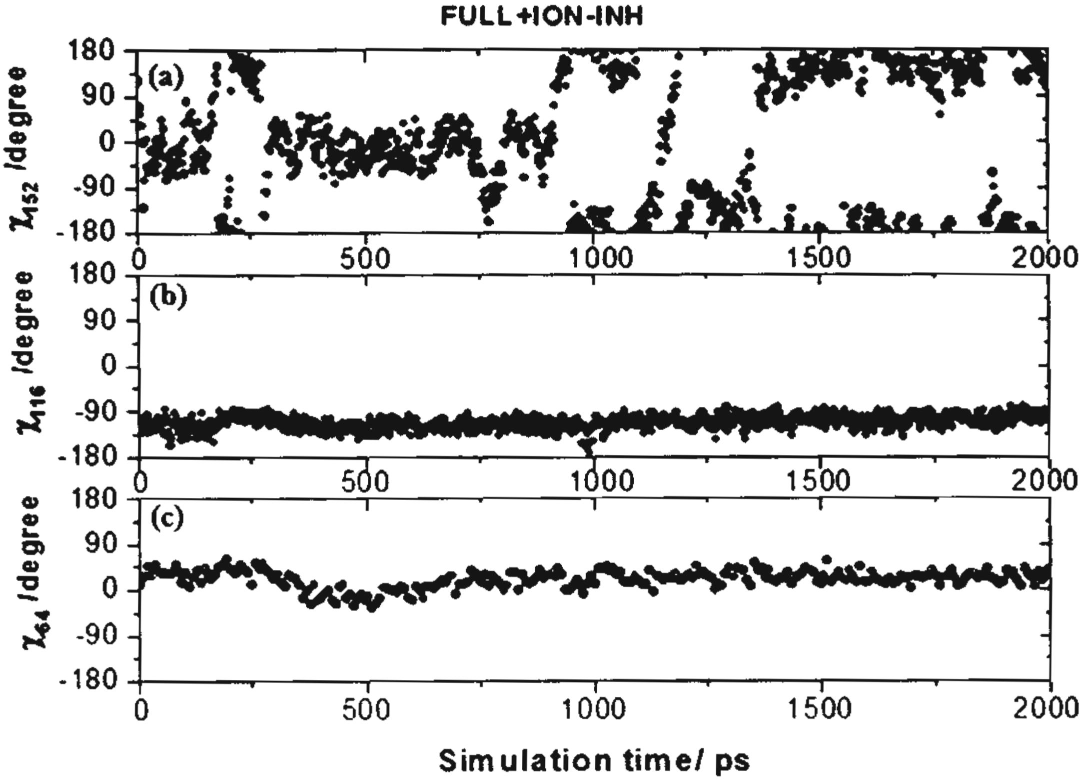


FIGURE 8 Torsional angles (χ_a) of the catalytic triad (a) χ_{152} (b) χ_{116} and (c) χ_{64} throughout the simulation of the FULL+ION-INH system.

Table 1 Detailed information for the two simulations of the systems complexed with 5-CITEP inhibitor, full-length (FULL+ION-INH) and the core domain (CORE-INH), number of water molecules, dimension of simulation boxes and number of total atoms,.

System	No. of ion		No. of water molecule	No. of atom	Dimension of the simulation box (Å^3)
	cations	Cl^-			
FULL+ION-INH	Mg^{2+}, Zn^{2+}	6	15423	50558	109.70 x71.75 x 64.48
CORE-INH	Mg^{2+}	-	7361	24577	68.36 x 64.27 x 55.97

Table 2. Distances between the Mg^{2+} and the carboxyl oxygen of Asp64 and Asp116; *d1*, *d2*, *d3* and *d4*.

Structure / chain	*d1*	*d2*	*d3*	*d4*	Coordination number of Mg^{2+}		Domain	Method	Ref.
					IN	Water*			
FULL+ION	1.91	1.92	1.86	3.89	3	3	Full-length	MD	29
CORE	1.93	1.93	1.87	3.97	3	3	Core	MD	29
FULL+ION-INH	1.95	1.94	1.87	3.53	3	3	Full-length	MD	This stud
CORE-INH	1.89	1.94	1.90	1.93	4	2	Core	MD	This stud

Table 3 The minimum, maximum and average distances between Mg^{2+} and Cα atoms of the missing residues, 140-149, in the catalytic loop, calculated during the MD simulation of the FULL+ION-INH and CORE-INH systems. Those values from previous studies were also reported.

Residue	Luca[37] (Subunit A)			FULL+ION[29]			FULL+ION-INH			CORE-INH		
	min.	max.	avg.	min.	max.	avg.	min.	max.	avg.	min.	max.	avg.
Gly140	9.75	21.97	15.34(±2.67)	13.81	20.08	17.13(±1.29)	10.93	13.98	12.40(±0.55)	8.47	13.47	11.52(±0.57)
Ile141	12.63	24.54	17.97(±2.36)	14.87	21.19	18.36(±1.45)	13.21	16.88	15.24(±0.54)	7.99	11.63	10.17(±0.37)
Pro142	15.04	24.11	18.32(±1.86)	17.34	21.09	19.42(±0.65)	15.40	18.39	17.00(±0.46)	11.04	14.19	12.58(±0.36)
Tyr143	16.11	26.90	21.28(±1.83)	14.85	17.28	16.08(±0.39)	12.95	16.23	14.59(±0.48)	12.17	15.37	13.62(±0.47)
Asn144	17.53	25.42	20.69(±1.59)	12.25	15.93	13.95(±0.62)	9.51	13.04	11.27(±0.47)	13.02	16.02	14.39(±0.42)
Pro145	16.39	24.51	20.26(±1.43)	12.37	16.79	14.91(±0.72)	11.62	15.36	13.49(±0.54)	14.32	18.94	16.49(±0.79)
Gln146	12.69	20.88	16.78(±1.41)	12.22	15.59	14.00(±0.62)	12.47	16.19	14.72(±0.56)	12.88	18.17	15.45(±0.81)
Ser147	9.95	19.22	14.19(±1.61)	9.42	12.13	10.73(±0.39)	8.62	13.95	11.73(±0.75)	9.49	14.32	11.78(±0.77)
Gln148	6.57	16.31	10.75(±1.84)	6.86	10.61	8.72(±0.73)	7.59	10.64	9.18(±0.42)	9.92	13.45	11.57(±0.56)
Gly149	7.39	13.92	10.08(±1.09)	8.23	12.61	10.26(±0.86)	10.27	13.08	11.51(±040)	8.62	11.55	9.95(±0.38)

ภาคผนวกที่ 2

2. บทความที่เกี่ยวข้องกับโครงการวิจัยนี้ที่ตีพิมพ์เผยแพร่ในวารสารทางวิชาการระดับนานาชาติ จำนวน 1 บทความ

Atchara Wijitkosoom, Somsak Tonmunphean, Thanh N. Truong, and Supot Hannongbua, "Structural and Dynamical Properties of a Full-length HIV-1 Integrase: Molecular Dynamics Simulations", *Journal of Biomolecular Structure and Dynamics*, 23(6): 613-624, **2006**.

Journal of Biomolecular Structure & Dynamics, ISSN 0739-1102
Volume 23, Issue Number 6, (2006)
©Adenine Press (2006)

Structural and Dynamical Properties of a Full-length HIV-1 Integrase: Molecular Dynamics Simulations

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Abstract

The structural and dynamical properties of the complete full-length structure of HIV-1 integrase were investigated using Molecular Dynamics approach. Simulations were carried out for the three systems, core domain only (CORE), full-length structure without (FULL) and with a Mg^{2+} (FULL+ION) in its active site, aimed to investigate the difference in the molecular properties of the full-length models due to their different construction procedures as well as the effects of the two ends, C- and N-terminal, on those properties in the core domain. The full-length structure was prepared from the two experimental structures of two-domain fragment. The following properties were observed to differ significantly from the previous reports: (i) relative topology formed by an angle between the three domains; (ii) the cavity size defined by the catalytic triad, Asp64, Asp116, and Glu152; (iii) distances and solvation of the Mg^{2+}; and (iv) conformation of the catalytic residues. In addition, the presence of the two terminal domains decreases the mobility of the central core domain significantly.

Key words: Molecular dynamics; HIV-1 integrase; Full-length; Structure.

Introduction

HIV-1 integrase (IN), a 288 amino acid residues protein, catalyzes an integration of the viral DNA into a host chromosome, an important step for propagation through the viral life cycle. The integration process consists of two main chemically reactions namely the 3′-processing and strand transfer (1). Integrase can also catalyze the disintegration reaction, which is the reversal of the integration step, *in vitro* (2). The proteolysis study shows that the integrase is composed of three parts: the central region (core domain) and two terminal ends (N-terminal and C-terminal domains) (1, 3, 4). It was reported that the core region is sufficient only for the disintegration but all three regions are required for the integration process (2). Consequently, the overall structure composing of all three domains will provide a potentially powerful target for rational design of drugs inhibiting this process. However, to the best of our knowledge, experimental data on the overall (full-length) structure is still not available. The domain full-length structures are shown in Figure 1a. The available experimental structures are only on each individual domain (the core, N-terminal, and C-terminal domains) and two-domain fragments (the core connected to the N-terminal and the core connected to the C-terminal) using X-ray crystallography and NMR techniques (5-11).

The lack of the experimental information on its complete three-dimensional structure has led to several proposed full-length structures (10, 12-16). In particular, there are three full-length structures suggested in the literature by Luca *et al.* (14), Podtelezhnikov *et al.* (15), and Karki *et al.* (16). Each used a different procedure for constructing the full-length model from the available experimental two-domain fragments. Podtelezhnikov *et al.* proposed the first full-length HIV-1 IN tetramer in

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which the full-length monomer was also built from individual domains. However, the domains from Podtelezhnikov *et al.*'s structure are not completely connected, *i.e.*, structures for the two linkage regions (residues 48-55 and 210-218) are still missing. The divalent metal ions were not included in this model structure (15). Luca *et al.* built the full-length HIV-1 IN by attaching individual domains using the core alone structure with the two end terminal domains from the two-domain fragment crystal structures, respectively. Two Mg^{2+} ions were included in the proposed structure (14). Karki *et al.* (16) proposed three models of the HIV-1 IN full-length that are also based on the two-domain fragment, 1EX4, in which the N-terminal domain was taken from different crystal structures (1WJA and 1K6Y). These suggested full-length models (10, 12-14, 16) have consistent relative arrangement of the three domains except that from Podtelezhnikov *et al.* (15) (see Figure 1b).

A closer examination was made to the structures provided by Luca and Podtelezhnikov. The results reveal noticeable differences. For instances: (i) Relative topology of the C-, core-, and N- domain, defined by the *a*-*b*-*c* angles where *a*, *b*, and *c* are, respectively, the center of mass of the three domains, is considerably different. Those values for the Luca's and Podtelezhnikov's models are 28° and 76°, respectively. (ii) Significant difference was found in terms of size of the catalytic cavity, defined by distances (*R*) between C atoms of the $-COO^-$ groups of the catalytic triad, Asp64, Asp116, and Glu152. The differences in the $R_{Asp64-Asp116}$, $R_{Asp64-Glu152}$, and $R_{Asp116-Glu152}$ obtained from the two models are 2.13 Å, 4.51 Å, and 5.93 Å, respectively. (iii) Orientation of each catalytic residue of the Luca's and Podtelezhnikov's models is almost incomparable. This is described by the rotation around the C_i-C_j bond of the side chain, $-C_i-C_j-COO^-$, of the catalytic triad. The differences are 10.9°, 161.8°, and 80.7° for the Asp64, Asp116, and Glu152 residues, respectively. Such large discrepancies of the structural parameters, especially those relate with the catalytic triad, extracted from the two available models and demonstrated in (i)-(iii) are known to effect ligand/enzyme interaction drastically. Therefore, questions arise on the reliability of the results when the two models were used in drug screening and design. Note that the question of how do these differences manifest in the dynamics of HIV-1 IN has not been examined.

There have been several studies using a Molecular dynamics (MD) simulation technique to provide useful information on the dynamical behaviors of this enzyme and insight into the enzyme-inhibitor complex behavior (17-20). Lins *et al.* performed the MD studies of the core domain alone in the presence and absence of the cation in the active site (18). Furthermore, the role of mutation on the inhibitor binding was studied by Barreca *et al.* (17). However, only the central core domain was considered. Moreover, the MD simulation of the whole full-length HIV-1 IN performed by Karki *et al.* (16) was just to get the lowest energy conformer. Recently, Luca *et al.* (21) have performed an MD study on the full-length-DNA complex aimed to understand the enzyme motion and the movement of the catalytic flexibility loop (residues 140-149). So far, the question of how one domain affects the structural and dynamical behaviors of the others in the complete system has not been addressed. Such information is important to understand the roles of each domain in the function of IN.

In the present study, our main goal is to determine whether a full-length IN is needed for drug design purpose. This requires us to address: (I) the differences in the structure and dynamics of the full-length HIV-1 IN models due to their different construction procedures; (II) the effects of other domains on the dynamics of the catalytic active sites in the core domain; and (III) the effects of a Mg^{2+} in the active site on the structure and dynamics of the protein in the active region. In doing so, we also constructed another full-length model using a different procedure than those previously employed. Comparisons to results from the previous structure (14-15) are provided. It is important to point out that HIV-1 IN is thought to a at least as a dimer in solution. The present study of the dynamics of the monome would provide a reference point to address the differences in the dynamics of the monomer and dimer in solution and how such differences manifest in its actual functions. Simulations on the full-length HIV-1 integrase dimer and its complex with DNA will be reported in a forthcoming paper.

Methods

Modeling the Full-length Structure of HIV-1 in Monomer

The full-length structure was built up using the two experimental crystal structures of two-domain fragments, the core connected to N-terminal (PDB code 1K6Y) (10) and the core connected to C-terminal domain (PDB code 1EX4) (11). The two-domain fragments were, for simplicity, named CORE-N and CORE-C, respectively. The CORE-N contains two missing regions, residues 47-55 and 140 -148, whereas the CORE-C has only one missing part, residues 142-144. The structures of CORE-N and CORE-C were superimposed using the core part as a common region. Then, residues 56-210 of the core domain of the CORE-C were removed. The peptide linkage between the residues Thr210 of the CORE-N and the Lys211 of the C-terminal was created and refined using Insight II (22). The reason for removing the core domain from the CORE-C rather from the CORE-N fragment in the superimposed structure is that the CORE-C contains more mutate residues. The obtained structure containing all three domains is referred to hereafter as FULL (Residue 1-270). Note that the residues (271-288) were not included in our full-length structure because they are still not resolved experimentally. It was reported that these residues do not play role in the DNA binding (23). The missing residues regions of the FULL structure were modeled and refined using homology modeling module in Insight II. Hydrogen atoms were added to the protein using the leap module in AMBER7 (24). In addition, the ionization state of the ionizable residues (*i.e.*, Asp, Glu, Arg, Lys, and His), and the charges at N-terminal residue, Phe1, and C-terminal residue, Asp270, were taken into consideration. All newly added residues (47-55, 140-148, 210-211) were relaxed in order to eliminate bad atomic contacts. The Mg^{2+} and Zn^{2+} cations were then placed in the core and N-terminal domains of the FULL in the same relative positions as found experimentally (7, 10). This structure is referred hereafter as FULL+ION.

MD Simulations

Both FULL and FULL+ION structures were solvated in rectangular boxes of water. Numbers of Cl^- ions were added in order to keep the overall system neutral. For each of the solvated FULL and the FULL+ION systems, 2 ns MD simulation was carried out using the AMBER7 package with the new force field of Carlos Simmerling *et al.* (25). Characteristics of the simulated systems are summarized in Table I. The following partial relaxations were applied while keeping the remaining structure fixed: first the hydrogen atoms, the newly added residues, and then the solvent molecules. The energy of the whole system was minimized by 100 steps using the steepest descent method followed by another 9900 steps using the conjugate gradient method. The system was thermalized from initial 10 K to the temperature of 300 K over 50,000 iterations (500 ps) and then equilibrated for 1 ns. The Particle Mesh Ewald (PME) method was used to calculate the full electrostatic interaction with 10 Å was cut off.

Table I

Number of water molecules, dimension of simulation boxes, and number of total atoms in the three simulations, full-length with (FULL+ION) and without (FULL) cations and the core domain only (CORE) systems.

System	No. of cations	No. of Cl^- ion	No. of water molecule	No. of atom	Dimension of the simulation box ($Å^3$)
FULL	-	3	12435	41562	103.07x64.74x77.69
FULL+ION	Mg^{2+}, Zn^{2+}	7	12854	41940	104.00x66.05x77.69
CORE	Mg^{2+}	1	7104	23780	68.705x69.842x63.404

(a)

N-terminal domain	Core domain	C-termina domain
HTH fold (HHCC)	Rnase fold (DD35E)	SH3 fol
1 50	51 212	213 2

(b)

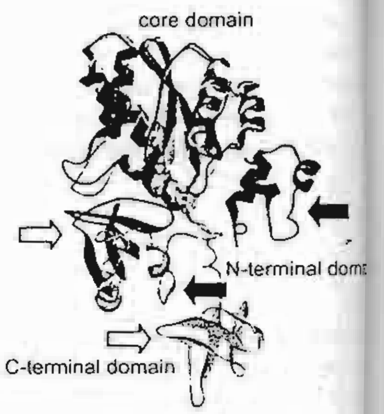

Figure 1: (a) Schematic representation of domains in HIV-1 IN and (b) superimposition of full-length model proposed by Luca *et al.* (green) Podtelezhnikov *et al.* (blue).

To figure out the effects of other domains on the dynamics of the catalytic active sites in the core domain, simulation of the CORE, core domain only (residues 50-209, 1BL3) including a Mg^{2+}, was additionally performed. Detailed information for this system was also given in Table I.

The MD simulations were carried out in an isobaric-isothermal ensemble (NPT) with a constant pressure of 1 atm and the temperature of 300 K. The external coupling bath26 with coupling constant of 0.2 ps was employed in order to keep the pressure constant throughout the simulations. A 2-fs time step was applied with SHAKE to constraint all bonds involving hydrogen atoms. Snapshots of coordinates of enzyme structure and water molecules were collected every 1 ps along the 2 ns MD simulations yielding 2000 frames stored for the trajectory analyses. The trajectories of both systems, FULL and FULL+ION, were analyzed using the PTRAJ and CARNAL modules in AMBER7. Three dimensional structures of the enzyme were visualized using the programs Molmol (27), Rasmol (28), and Swiss-PDB Viewer (29). The quality of the protein structures were evaluated using the Procheck program (30).

Results and Discussion

Dynamical Stabilities

Stability of the systems was examined by monitoring their thermodynamic properties. Figure 2 shows energies and temperature profiles for 2-ns simulation of the FULL and FULL+ION systems. The results indicate reliability and stability of the simulated systems. The average temperatures for the simulations with and without the ions are 300K.

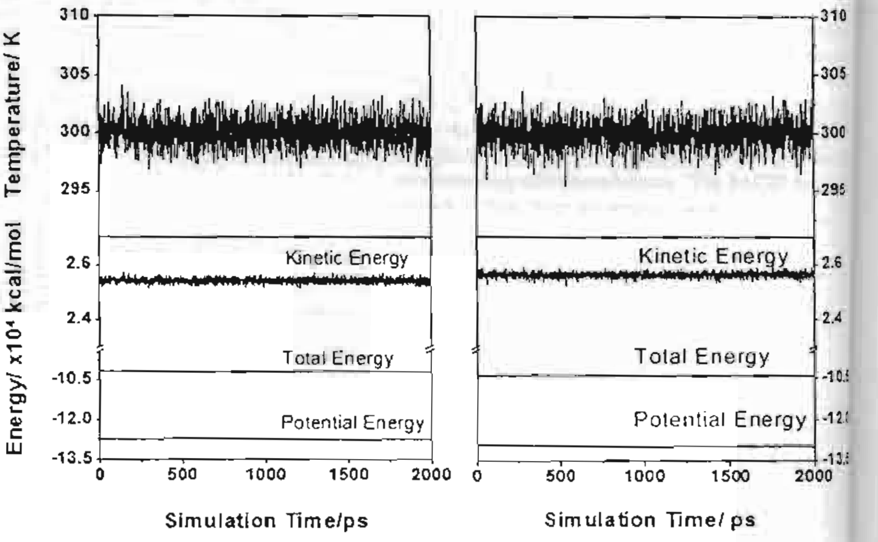

Figure 2: Energy and temperature profiles for (a) the FULL and (b) the FULL+ION over the 2-ns simulation time.

Dynamical Behaviors of the Full-length HIV-1 IN

The root-mean-square deviations (RMSD) of the Cα atoms with respect to th average structure over the 2-ns simulation time of the FULL+ION and the FUL were evaluated and shown in Figure 3a, the RMSD for the three core domain were given in Figure 3b, while these values per residue were also investigated an plotted in Figure 3c. Throughout the simulation, the FULL+ION is, as expecte less flexible than the FULL systems. The RMSDs in Figure 3a are fluctuated i the range 1.25-3.11 Å for the solvated FULL+ION system and 1.24-3.60 Å for t FULL system. This observation can be understood in terms of ion-ligand bindi which restricts motions of residues in the vicinity of the ion.

Effect of the two end domains (C-terminal and N-terminal) on the mobility of t central core region can be clearly seen from the RMSD plots shown in Figure 3b which the movement of the CORE (RMSD = 1.73 Å) is higher than those of the core domains of the other two full-length systems (RMSD ≈ 1 Å). Rigidity of the core regions in the full-length structures can be due to their bindings with the two ends.

The RMSD plotted per residue in Figure 3c demonstrated the high flexibility of the two ends of both systems, FULL and FULL+ION, especially at the C-terminal, the DNA binding domain. Such large fluctuation of the C-terminal is due to the long linker (about 10 amino acid residues) between the core and the C-terminal. Consider the core only regions of the three systems (residues 56-209), the RMSD of the CORE in the two regions, 188-192 and 207-209, are considerably higher than those of the two full-length systems.

Flexibility of the Elbow Linkage in the Two Terminal Regions

From our simulations, we observed such dynamical behavior of the elbow linkage region. The structures were snapshoot every 100 ps from the 2 ns trajectory were superimposed, the RMSD per residue were also calculated (data not shown). High flexibilities of the residues 225-270 were clearly observed. The flexibility of the elbow region was confirmed by the RMSD plot over simulation time of the system (Figure 4). Each domain fluctuates constantly and has lower magnitude than that of the full-length one. This character was also found in the linkage between the core and the N-terminal domain (residues 47-55) in which still being poorly define region.

The linkage between the central core and the N-terminal domain, residue 47-55, and the central core and the C-terminal domain, residues 195-220, were reported to be flexible (10-11). The linkage region in the N-terminal part was resolved in the N-terminal structure from NMR studies (5) whereas this region was still missing in the crystal structure of the CORE-N (10). This region (residues 47-55) in the N-terminal structure was a long straight coil while it was connected to the core domain the modeled region in the full-length structure. The MD results display the high flexibility of this linkage region as we can see that the RMSD per residue of the N-terminal region has high value whereas the RMSD over simulation time of this domain was stable at ~0.8 Å (Figures 3 and 4).

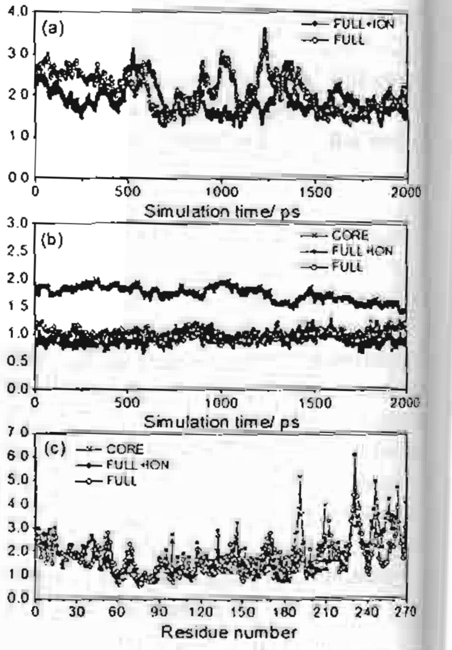

Figure 3: RMSDs of the Cα of the HIV-1 IN with respect to the average structure as a function of the simulation time plotted for (a) the whole full-length system with (FULL+ION) and without (FULL) the ions and (b) only for the core regions of the FULL, the FULL+ION, and the CORE systems, calculated by fitting onto its corresponding reference structure. The RMSD for each residue of those three systems are in (c).

Similar to what found in the N-terminal domain region, the linkage region involving in the C-terminal region (residue 195-220) shows high flexibility (Figure 3) while the RMSD over 2-ns simulation time was rather stable and lower than that of the full-length (Figure 4). The results are in the same manner for both the FULL+ION and the FULL. The region in the C-terminal part was found to have a kink at residue Thr210 in one monomer of the CORE-C (11) structure and thus present in our full-length model. A previous study suggested that this flexibility may play a key dynamical role in multistep integration process (31). Comparison were also made to the core-c domain among the different IN structure, *i.e.*, RSV, SIV, and HIV-1, it was found that the orientation of these regions were different (32-33). These observations support the functional flexibility in this region, which is proposed to help in tethering the DNA during strand transfer process (11). Compared to MD simulation by Luca (21), it is obviously seen that the RMSD of the DNA-dimer full-length complexed system revealed higher mobility than our system. Such high flexibility is not yet understood. However, this can be due to the interaction among the two monomers and the DNA in the system.

Full-length HIV-1 IN: Arrangement of the Three Domains

First, we analyze how different our full-length HIV-1 IN structure is compared to the two existing structures from Podtelezhnikov *et al.* and Luca *et al.* by superimposing these structures (see Figure 5). Substantial differences were found between our FULL (red) and Podtelezhnikov's (blue) structures, especially at the two ends (Figure 5a). Our full-length structure is generally in good agreement with that

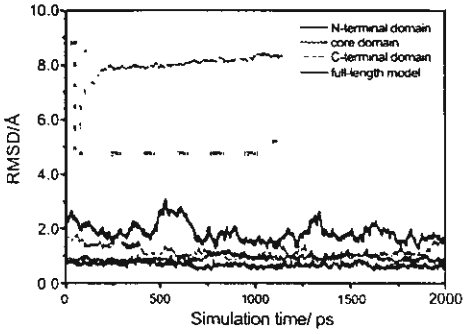

Figure 4: RMSDs of the Cα with respect to the average structure over 2-ns simulation time. The RMSD values of each domain, N-terminal, core, C-terminal, and the overall full-length structure were separately calculated by fitting onto its corresponding reference structure. MD result published by Luca (21) was also shown as an inset.

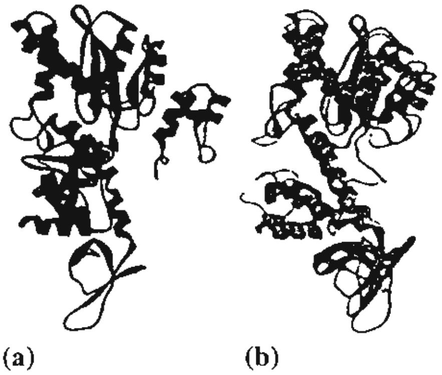

Figure 5: Superposition of our average MD structures (violet and red) with (a) Podtelezhnikov's (blue) and (b) Luca's (green) models for the full-length HIV-1 IN without (FULL) and with (FULL+ION) Mg^{2+}.

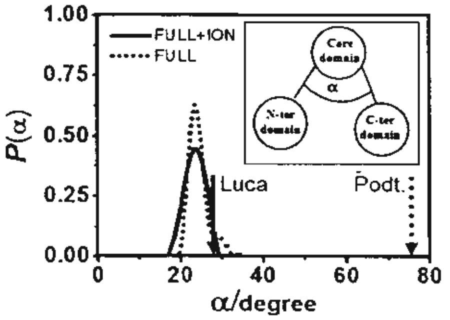

Figure 6: Probability distribution of the angle α representing relative orientation of the core, N-terminal, and C-terminal domains of our full-length HIV-1 integrase, FULL+ION (thick line), and FULL (thin line). The arrows are the corresponding values calculated from the Podtelezhnikov's and Luca's models.

of Luca's (green) with some differences found in the residues 47-55 and the C terminal (Figure 5b). These differences are due to the different procedures used i synthesizing the starting full-length structure. Our full-length HIV-1 IN was buil up using the CORE-N and CORE-C structures whereas Luca *et al.* also used th two-domain fragments but the synthesizing procedure is different from ours, *i.e.,* Luca *et al.* started the process using the one-domain core structure (1QS4), then added the N- and C-terminal domains to form the full-length by superimpositio the conserved core domain of the two two-domains (CORE-N and CORE-C) t the 1QS4 core structure. Podtelezhnikov and co-workers modeled the full-lengt HIV-1 IN by combining three individual separated domains taken from the Protei Data Bank and left the linker residues (48-55 and 210-218) blank, *i.e.*, the overal structure was not completely connected.

Our results indicated that there is no significant difference among the four model (our FULL and FULL+ION, Podtelezhnikov's and Luca's models) in terms o geometry and orientation within each domain. This is not surprising since eac single domain structure was taken from x-ray data. However, as indicated abov there are differences in the relative orientations of the three separated domains, co connected to N-terminal and core connected to C-terminal. To elucidate this aspe more quantitatively, relative orientations between the core and the two end domain were measured in terms of the angle α, formed by the two vectors pointing from to *b* and *a* to *c*, where *a*, *b*, and *c* are the averaged locations from all atoms of th core, N-terminal, and C-terminal domains, respectively. The distribution of th angle α extracted from the MD simulations was displayed in Figure 6. The sam angle was also calculated from Podtelezhnikov *et al.*'s and Luca *et al.*'s models an the values were also plotted in the same figure for comparison.

The distribution plot for the FULL+ION shows the α fluctuated in the range 18° 27° with the average value of 23°, whereas, that of the free form, FULL, yields th fluctuation range of 19°-31° and the average value at 24°. The corresponding value for the angle α are 76° and 28°, for the Podtelezhnikov *et al.*'s and Luca *et al.*' models, respectively. The results indicate that our models are consistent with thos from Luca *et al.* whereas those from Podtelezhnikov *et al.* show a large deviatio This implies that generating of the full-length structures from the two connecte CORE-N and CORE-C domains may provide more consistent structures than th from the separated core, N-terminal, and C-terminal domains.

Structure of the Missing Regions

Our full-length model confirms the previous report of Wang *et al.* that ev though the arrangements of the two crystal structures of the two-domain fragme structures are different, *i.e.*, the structure of the CORE-N is quite compact where the CORE-C is more extended, the superimposition of these two structures w

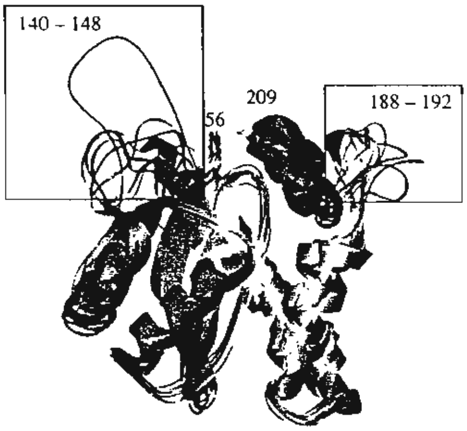

Figure 7: Superimposition at the co domain of nine structures, four full-len structures; average MD of FULL+IO (black) and FULL (brown) in our stu models proposed by Podtelezhnikov (oran and Luca (red), three average MD of tw domain structures (our data in preparatio CORE-N (yellow), CORE-C (gree and CORE (cyan), and three crystal c structures: 1BIS chain B (blue), 1BI3 ch C (cyan), and 2ITG (gray). Numbers of first and the last residues (56 and 209), missing residues (140-148) and the flex regions (188-192) of the core domain w labeled. In addition, the two flexible regi of the core domain were also enlarged.

well ordered with no steric clash (10). The linker, residues 47-55, is mobile and disordered in the crystal structure. It was found to form a random coil, similar to that observed in the solution structure of the N-terminal domain (5). In earlier works, the core domain, residues 50-212, is known to often have two missing regions, residues 140-148 and 188-192. Among the crystal structures of the core domain available to date, there are three structures in which the former region was solved; 1BIS chain B, 1BL3 chain C, and 2ITG (7-8, 34). The latter region has already been solved in many crystal structures. This suggests that structures in these two regions, residues 140-148 and 188-192, are rather flexible.

As for the experimental availability of the two-domain fragment structures, the CORE-N and the CORE-C, these two structures are of importance for the modeling of HIV-1 IN full-length and were used in almost previous studies (14-17). Other 2-ns MD simulations of the CORE-N and CORE-C including the cations in the active site were also performed (data not shown here). The results confirm the high flexibilities of these regions, residues 140-149, residues 188-192, and the two ends (N-terminal and C-terminal). The coordination of three carboxyl oxygen atoms from enzyme and three water molecules were found.

Consider the catalytic core regions, the core component of ten structures, four average MD structures of FULL+ION, FULL, CORE-N and CORE-C, two full-length structures proposed by Podtelezhnikov *et al.* and Luca *et al.*, and the above three crystal structures, were superimposed and compared as shown in Figure 7. The results confirm that structures of the core domain are well conserved except for the two regions (140-148 and 188-192) as mentioned above that have large deviations amongst these structures. The residues 140-148 in our simulated structures vary in the same range of other structures except for that of 2ITG, the long extended coil. Likewise, residues 188-192 show large differences between these nine structures. Furthermore, the protein structures in this region in Podtelezhnikov *et al.*'s and Luca *et al.*'s models and in the CORE-C experimental structure are greatly different from the rest.

The dynamical behaviors of the missing region, residues 140-149, were investigated to explore the conformational change of this region. The results can be directly compared with those of subunit A of Luca's simulation (21) (see Table II). The differences among the two models were measured and plotted in Figure 8. The investigated distances as well as the corresponding standard deviations obtained from our simulation were distributed in a narrower range, the differences between the maximum and the minimum distances, in comparison to those of Luca's work (21). This confirms the RMSD plots shown in Figure 4 in which Luca's values are higher than 8 Å. The plot in Figure 8 indicates that our missing loop approaches the Mg^{2+} closer than that of subunit A of Luca's.

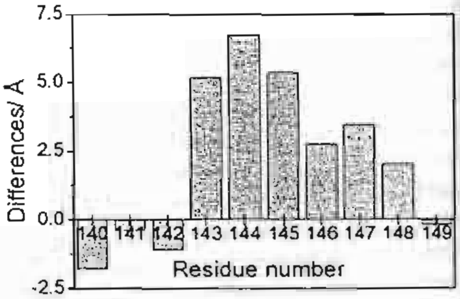

Figure 8: Differences, Luca's subtract by our values, between the average Mg^{2+} - Cα distances of the missing residues (140-149) in the catalytic core domain.

Tyr143 in the active site was suggested to play important role in the catalytic activity of the integrase (35). Luca reported marked different movement of Tyr143 in the

Table II
The minimum, maximum, and average distances between Mg^{2+} and Cα atoms of the missing residues, 140-149, in the catalytic loop, calculated during the MD simulation.

Residue	Subunit A[21]			This study (FULL+ION)		
	min.	max.	average	min.	max.	average
Gly140	9.75	21.97	15.34 (±2.67)	13.81	20.08	17.13 (±1.29)
Ile141	12.63	24.54	17.97 (±2.36)	14.87	21.19	18.36 (±1.45)
Pro142	15.04	24.11	18.32 (±1.86)	17.34	21.09	19.42 (±0.65)
Tyr143	16.11	26.90	21.28 (±1.83)	14.85	17.28	16.08 (±0.39)
Asn144	17.53	25.42	20.69 (±1.59)	12.25	15.93	13.95 (±0.62)
Pro145	16.39	24.51	20.26 (±1.43)	12.37	16.79	14.91 (±0.72)
Gln146	12.69	20.88	16.78 (±1.41)	12.22	15.59	14.00 (±0.62)
Ser147	9.95	19.22	14.19 (±1.61)	9.42	12.13	10.73 (±0.39)
Gln148	6.57	16.31	10.75 (±1.84)	6.86	10.61	8.72 (±0.73)
Gly149	7.39	13.92	10.08 (±1.09)	8.23	12.61	10.26 (±0.86)

two subunits and observed conformational changes of subunit B after 250 ps (see an inset of Figure 9). Distances between OH atom of the Tyr143 and the Mg^{2+} yielded from our simulation are slightly shorter than those of subunit A of Luca.

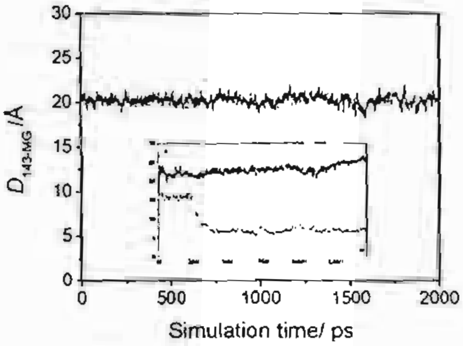

Figure 9: Dynamic profile of distance between atom OH of Tyr143 and the Mg^{2+} over the 2-ns simulation where that of Luca (21) (subunit A: black line and subunit B: grey line) was given as an inset.

Conformation of the Active Site

The active site region on the core domain, a DD35E motif, contains two aspartic and one glutamic residues. The Mg^{2+} cation is bound to these residues. The coordination of the ion and two conserved aspartate residues is shown in Figure 10.

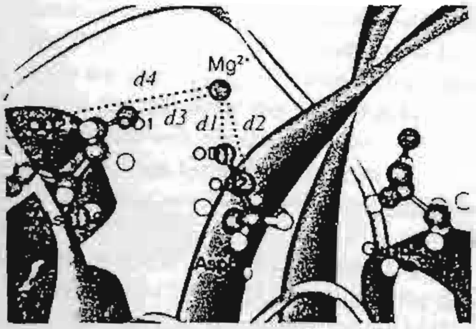

Figure 10: Schematic representation of the catalytic region of the core domain of the HIV-1 IN consisting of a Mg^{2+}, the two aspartate residues (Asp64 and Asp116), and a Glu152 for the FULL+ION simulation.

To understand the structure of the catalytic region, especially how the presence of the Mg^{2+} cation as well as the two ends affects the cavity for inhibitor binding of this region, distributions of the selected distances and angles of the three systems (FULL+ION, FULL, and CORE) over the simulation time were evaluated and plotted in Figures 11-13. Corresponding values were also extracted from Podtelezhnikov *et al.* and Luca *et al.* structures and were also plotted for comparison. Note that the distances, $R_{x\text{-}y}$, were measured from a carboxyl carbon atom in the –COO– groups of residue x to the same type of atom of residue y where subscripts x and y can be Asp64, Asp116, or Glu152 (Figure 11). The coordination of the cation in the active site and the torsional angles were defined in an inset of Figures 12 and 13.

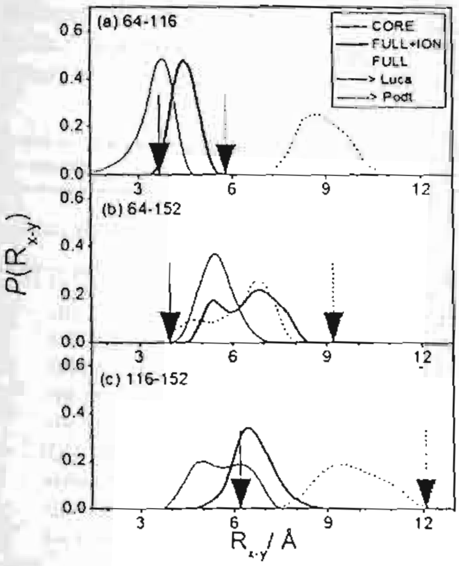

Figure 11: Distribution of the distances $P(R_{x\text{-}y})$ between each catalytic triad (see text for details): Asp64-Asp116 (a), Asp64-Glu152 (b), and Asp116-Glu152 (c) throughout the 2-ns simulations for the CORE (thin line), FULL+ION (solid line), and the FULL (dash line). Single value extracted from Podtelezhnikov's (dash arrow) and Luca's (solid arrow) models were also displayed.

For the $R_{64\text{-}116}$ (Figure 11a), the FULL+ION system has a single sharp peak at 4.25 Å (solid line) due to the tight binding between the Mg^{2+} and two aspartate residues, *i.e.*, both residues were moved consistently together resulting in the narrow distribution of its distance. In contrast, the FULL system showed a broad peak (dash line) indicating more flexibility. In the case of $R_{64\text{-}152}$ (Figure 11b), both the FULL+ION (solid line) and the FULL (dash line) systems displayed broad distribution patterns with no prominent peak. The reason may be due to the rather flexible Glu152 residue indicated by its torsion angle as discussed in more detail below while the Asp64 is rather rigid since it is bound to the Mg^{2+}. Considering the $R_{116\text{-}152}$, the FULL+ION (Figure 11c, solid line) showed a broad single peak at around 6.25 Å. This distance in the FULL system (Figure 11c, dash line) is, as expected, shifted to a longer distance and showed a broader peak than that of the FULL+ION. In comparison to the available data, our distances of the FULL+ION system are in agreement with those reported by Luca *et al.*, except for $R_{64\text{-}152}$. On the other hand, the FULL system exhibits substantially different distances from those of the Podtelezhnikov *et al.* model, especially the $R_{64\text{-}116}$ distance.

Effects of the two ends on the catalytic size were also exhibited by the distribution plots shown in Figure 11. The $R_{64\text{-}116}$ distance of 3.93 Å for the CORE system is substantially shorter than that of 4.25 Å for the FULL+ION structures (Figure 11a). The $R_{64\text{-}152}$ for the CORE shows single peak at 5.45 Å while that of the FULL+ION appears two preferential distances, 5.25 Å and 6.75 Å (Figure 11b). This indicates much lower rigidity of this distance of th CORE than those of the FULL and FULL+ION systems. Situation is opposit for the $R_{116\text{-}152}$ where the CORE favors two conformations. The above finding lead to a clear conclusion that the presence of the two ends affects the size and flexibility of the catalytic site of the core domain.

In terms of the cation's coordination, distributions of the distances between electron donor atoms of the catalytic residues and the Mg^{2+}, *d1*-*d4* shown in Figure 8, for the three systems were plotted in Figure 12. Comparisons with the available data, the cation's coordination from the three crystal structures (each composes of three monomers in the unit cell) of the core domain only; 1BIU, 1BL3, and 1QS4 and from the simulation results by Lin *et al.* were also summarized in Table III. It can be seen from Table III that the X-ray data of the three monomers, as well as the simulation results by Lin *et al.* (16) predict that the Mg^{2+} coordinates with four oxygen atoms from the water molecules and two carboxyl oxygen atoms of the Asp64 and Asp116. The experimental Mg^{2+}-O distances are in the range 2.10-2.91 Å while those of Lin *et al.* were not reported.

For the Luca *et al.* dimer full-length structure where two Mg^{2+} ions per monomer were taken into account (Table III), the coordination number was not reported. However, with an assumption that binding could be formed when the Mg^{2+}-O distance is shorter than 3 Å, we measured all the Mg^{2+}-O distances in their modeled structure and found that the coordination number can be either three or four. The first Mg^{2+} of chain A coordinates with four oxygen atoms of Asp64 and Asp116 while the second Mg^{2+} of chain A forms three bonds with oxygen atoms, one from Asp64 and the other two from Asp116. In contrast, for chain B, the first Mg^{2+} coordinates with one oxygen atom of Asp64 and two oxygen atoms of Asp116 and the second ion forms 4 bonds with four carboxyl oxygen atoms, two from Asp116 and the other two with Asp64 (see Table III).

From the distribution plot (Figure 12) and the optimal Mg^{2+}-O distances (maximum of the peak) (Table III), our results for the CORE and FULL+ION also indicate that the coordination number for the Mg^{2+} is six but the specific binding structure is different from the previous studies. The octahedral sites of our data of the Mg^{2+} contain two oxygen atoms of Asp64, one oxygen atom of Asp116, and three oxygen atoms of the water molecules. However, our solvation properties are, quantitatively, in the range obtained from the MD results of the full-length structure by Luca *et al.* that observed 1-4 bonds between the Mg^{2+} and the catalytic residues and the corresponding Mg^{2+}-O distances in the range of 2.06-2.99 Å. It should be noted that three coordinations with enzyme were also found in subunit

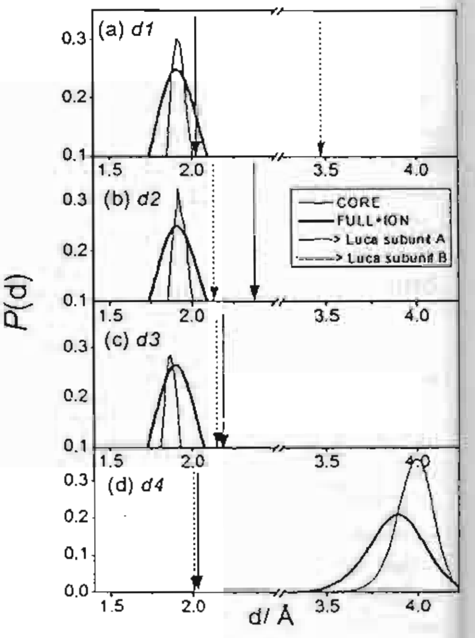

Figure 12: Distribution of the distance P(*d*) between Mg^{2+} and the carboxyl oxygen of the catalytic triad residues, *d1*, *d2*, *d3*, and *d4*, as for Asp64:OD1, Asp64:OD2, Asp116:OD1, Asp116:OD2, respectively, for the CORE, FULL+ION, and the FULL systems. The arrows illustrate the data extracted from the two subunits of Luca's structure.

Table III

Distances between the Mg^{2+} and the carboxyl oxygen of Asp64 and Asp116; *d1*, *d2*, *d3*, and *d4* where are the three monomers in the unit cell.

Structure / chain	*d1*	*d2*	*d3*	*d4*	Coordination number of Mg^{2+}		Domain	Method	Ref
					IN	Water*			
1BIU (1)	3.57	3.87	5.07	4.45	-	*n*	core	X-ray	7
1BIU (2)	2.31	3.66	3.63	2.10	2	*n*			
1BIU (3)	2.82	3.53	3.90	2.30	2	*n*			
1BL3 (1)	2.42	3.78	4.01	2.39	2	4	core	X-ray	8
1BL3 (2)	2.34	3.30	4.34	2.91	2	*n*			
1BL3 (3)	2.20	3.53	3.93	2.32	2	4			
1QS4 (1)**	2.32	3.54	2.25	3.77	2	4	core	X-ray	9
1QS4 (2)	2.37	3.49	2.34	3.92	2	4			
1QS4 (3)	2.59	3.69	2.43	3.86	2	*n*			
Lin *et al.*	*n*	*n*	*n*	*n*	2	4	core	MD	17
Luca*** / A ion1	2.04	2.38	2.17	2.09	4	*n*	Full-length	Mini-mization	14
ion 2	4.21	2.11	2.13	2.12	3				
Luca / B ion 1	3.47	2.10	2.11	2.06	3	*n*			
ion 2	2.10	2.99	2.16	2.14	4				
FULL+ION	1.91	1.92	1.86	3.89	3	3	Full-length	MD	This study
CORE	1.93	1.93	1.87	3.97	3	3	core	MD	This study

* *n*: not available; ** complexed with inhibitor; *** complexed with DNA.

B of Luca's structure. This is shown by the *d2*, *d3*, and *d4* of 3.47 Å, 2.10 Å, and 2.11 Å, respectively (dash arrows, in Figure 12).

It can be seen from the maxima of the distribution plots for the three distances to the carboxyl oxygen (*d1-d3*) (Figure 12 and Table III) that significant changes of the Mg^{2+}-O distances were not observed in comparison between the CORE and FULL+ION systems. However, all plots for the CORE are considerably narrower than those of the FULL+ION, indicating lower flexibility of the coordination of the former than the latter structures. Note that the distances of 3.97 Å for the CORE and 3.89 Å for the FULL+ION are too far to coordinate to the Mg^{2+}.

Some comments should be made concerning the *d1-d4* in which the distances observed from our study for both CORE and FULL+ION systems are notably shorter than those of the other works. Due to the environmental differences, the coordination distances obtained from the x-ray crystal structures, 1BIU, 1BL3, and 1QS4, and the simulation are usually incomparable. In addition, discrepancy between our and Luca's ones (see Table III) can be due to the different methods used, *i.e.*, Luca's structure was the results of the structure minimization. Moreover, our Mg^{2+}-O distances ranging from 1.86-1.93 Å agree with those of Mg^{2+} in aqueous solution of 2.0-2.05 Å obtained from the simulation using *ab initio* fitted potentials (36) and the QM/MM simulations (37).

In addition to the ion's coordination numbers and the related distances which represent the size of the catalytic site of the HIV-1 IN, binding affinity between the enzyme and the inhibitor was known to depend, obviously, on the orientation of the catalytic residues. Such information for the CORE, FULL, and FULL+ION was evaluated and displayed in Figure 13 in terms of torsional angles as defined in the inset. The plot indicates clearly that the presence of cation leads to dramatic changes of the orientation of the catalytic residues, especially for the Asp64 and Asp116. Without the Mg^{2+}, two preferential conformations of the Asp64 at the torsional angles 65° and -155° were observed (Figure 13a, dash line). The presence of the Mg^{2+} induces the two preferential conformations of the Asp64 in the FULL to give only one stable conformation at the torsional angles of 5° and 13° for the CORE and FULL+ION systems, respectively (Figure 13a, thin and dark solid lines). The angle of 41° was extracted from the structure published by Podtelezhnikov *et al.* and 30° by Luca *et al.*

For the Asp116 catalytic residue, the two preferential conformations in the FULL system (χ_{116} = -125° and 65°, Figure 12b, dash line) were shifted by the Mg^{2+} to form the most stable structure at -145° and another minor conformation at 155° (Figure 13b, dark solid line). This is different from the CORE system where only one stable conformation at -165° was observed (thin solid line in Figure 13b).

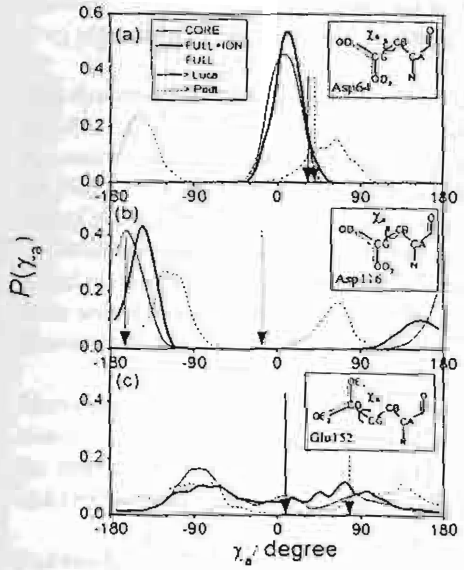

Figure 13: Distribution of the torsional angles (χ_a) of each catalytic triad: χ_{64} (a), χ_{116} (b), and χ_{152} (c), for the CORE (thin line), FULL+ION (solid line), and the FULL (dash line) systems. The values measured from Podtelezhnikov's (dash arrow) and Luca's (solid arrow) models were also plotted.

No preferential conformation was found for the Glu152. Its conformation is highly flexible in all CORE, FULL, and FULL+ION systems (Figure 13c). This is understandable because this residue is not located within the solvation shell of the Mg^{2+}. The detected data agree well with the previous report on the catalytic core domain by Lins *et al.* that stated that the Glu152 shows large fluctuations (18).

Conclusion

HIV-1 integrase (IN) is becoming a new promising target for the design of anti-AIDS drug. The integrase, catalyzes insertion of viral DNA into human chromosome, contains three structural domains, core, N-terminal, and C-terminal domains. Molecular dynamics simulations were investigated for the three model systems, core domain only (CORE), full-length structure without (FULL), and with a Mg^{2+} (FULL+ION) in its active site. This aimed to investigate the difference in the molecular properties of the full-length models due to their different construction procedures as well as the effects of the two ends, C- and N-terminal, on those properties in the core domain. In addition, the noticeable differences among previous models suggest further examinations into the dynamical properties of these full-length models are needed.

The full-length structure was prepared from the two experimental structures of the two-domain fragment, CORE-N and CORE-C using. The model was examined with those structures provided by Luca and Podtelezhnikov. The relative topology of the three domains in terms of the angle between the C-, Core-, and N- terminal of 23° obtained from our study is similar to that of 28° from Luca's model but differs from that of 76° from Podtelezhnikov's model. Discrepancies in the structural and dynamical properties defined by distances and conformation of the catalytic triad obtained from the three models using different construction procedures, were substantially observed.

The presence of the two ends (C-terminal and N-terminal) decreases the mobility of the central core domain significantly. The RMSDs for the CORE and full-length (FULL and FULL+ION) systems are 1.73 Å and ~1 Å, respectively. In terms of cavity size defined by the distances between the catalytic triad, Asp64, Asp116, and Glu152, the $R_{64\text{-}116}$ distance of 3.93 Å for the CORE system is substantially shorter than that of 4.25 Å for the FULL+ION structures. The $R_{64\text{-}152}$ for the CORE shows single peak while the FULL+ION prefers two conformations. The results show the effect is opposite for the $R_{116\text{-}152}$ where the CORE favors two conformations. The difference between those of the core-only and the full-length structure suggest us to conclude that to the full-length HIV-1 IN is needed for drug screening and drug design purpose.

The dynamical behavior, in terms of RMSD, of the FULL+ION is, as expected, less flexible than the FULL systems. The effects of a Mg^{2+} on the structure and dynamics of the catalytic residues were found. The $R_{64\text{-}116}$ and $R_{116\text{-}152}$ distances for the FULL+ION are much shorter and their flexibilities are lower in comparison to that of the FULL systems. However, no significant different was found for the $R_{116\text{-}152}$ distance and its flexibility of the two systems. The presence of the Mg^{2+} was also found to induce the two preferential conformations of the Asp64 and the Asp116 to form only one stable conformation. In addition, no preferential conformation was found for the Glu152 in both systems.

Moreover, we are currently studying the structure and dynamics of the HIV-1 IN dimer and tetramer binding to DNA in the presence of metal ions and inhibitor in the active site to provide understanding on the interaction between the HIV-1 IN and DNA. Results of such a study will be reported in a forthcoming paper.

Acknowldgements

The authors would like to acknowledge the Royal Golden Jubilee (RGJ) Ph.D. program and the Thailand Research Fund (TRF) for all funding. We thank for all computing facilities provided by the Computational Chemistry Unit Cell (CCUC) at Department of Chemistry, Faculty of Science, Chulalongkorn University. A. W. is grateful to the University of Utah for a short-term fellowship, the Henry-Eyring Center for Theoretical Chemistry and Center for High Performance Computing (CHPC) at the University of Utah for computer time and supports. We thank Professors Thomas E. Cheatham, III for AMBER parameters and James M. Briggs for valuable discussions and Luca *et al.* and Podtelezhnikov *et al.* for providing their model full-length coordinates.

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Date Received: October 9, 2005

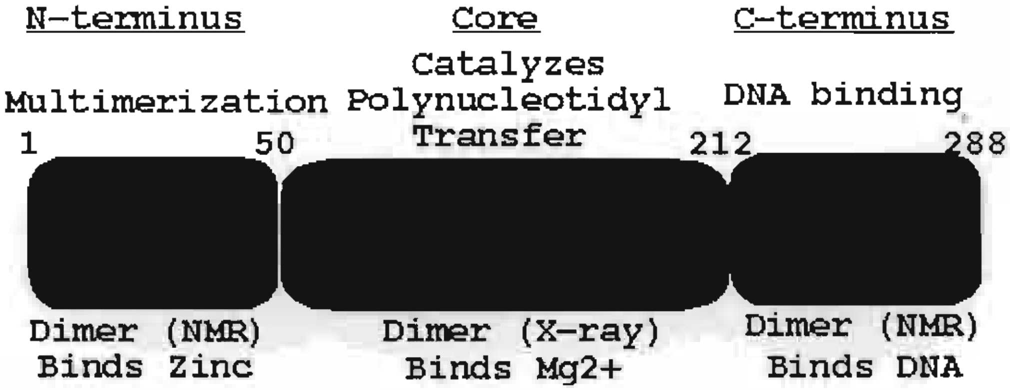
Communicated by the Editor Ramaswamy H. Sarma

ภาคผนวกที่ 3

3. บทความสำหรับการเผยแพร่

โรคเอดส์ (AIDS, Acquired Immune Deficiency Syndrome) หรือโรคภูมิคุ้มกันบกพร่องเกิดจากเชื้อ HIV (Human Immunodeficiency Virus) ซึ่งในวงจรชีวิตของเชื้อ HIV จำเป็นต้องอาศัยเอนไซม์สำคัญ 3 ชนิด คือ รีเวอร์สทรานสคริปเทส (reverse transcriptase), โปรติเอส (protease) และอินติเกรส (integrase) ในปัจจุบันยารักษาโรคเอดส์มุ่งเน้นอยู่ที่การยับยั้งการทำงานของเอนไซม์ 2 ชนิดแรก แต่มีการพบว่าเชื้อไวรัส HIV เกิดการดื้อต่อยาดังกล่าว รวมทั้งยาบางตัวยังมีผลข้างเคียงอีกมากมาย นักวิจัยทั่วโลกจึงได้ให้ความสนใจในการค้นหาตัวยับยั้งที่ตำแหน่งใหม่ ซึ่งได้แก่ที่ตำแหน่งเอนไซม์เอชไอวี-1 อินติเกรส โดยมุ่งหวังว่าสารยับยั้งในตำแหน่งดังกล่าวจะช่วยเพิ่มประสิทธิภาพในการรักษาโรคเอดส์ชนิดที่ดื้อต่อยาที่ใช้กันอยู่

โครงสร้างของเอนไซม์อินติเกรสประกอบด้วย 3 ส่วน (domain) คือ N-terminal domain, catalytic core domain และ C-terminal domain ดังแสดงในรูปที่ 1 โดยที่ส่วนสำคัญคือ core ซึ่งเป็นส่วนที่เข้าจับกับตัวยับยั้ง อย่างไรก็ตามปัจจุบันนักวิจัยสามารถหาโครงสร้างของแต่ละส่วนเดี่ยว ๆ ในสภาพอิสระของทั้ง 3 โดเมน, โครงสร้างของ N-terminal domain ต่อกับ catalytic core domain และโครงสร้างของ catalytic core domain ต่อกับ C-terminal domain ได้ แต่ยังไม่สามารถหาโครงสร้างของอินติเกรสที่สมบูรณ์ซึ่งประกอบด้วยทั้ง 3 โดเมนได้ ดังนั้นงานวิจัยที่มีอยู่ในปัจจุบันจึงทำการศึกษาโครงสร้างการเข้าจับโดยใช้เฉพาะโครงสร้างส่วน core เท่านั้น ซึ่งจะไม่สอดคล้องกับสภาพการทำงานจริงของอินติเกรสที่ต้องมีโครงสร้างครบทั้ง 3 ส่วน



รูปที่ 1 โครงสร้างของเอชไอวี-1 อินติเกรสแต่ละโดเมน

การขาดโครงสร้างที่สมบูรณ์ของเอนไซม์ รวมทั้งการขาดความรู้ความเข้าใจเกี่ยวกับกลไกการออกฤทธิ์ โครงสร้างการเข้าจับ และแรงกระทำ (interaction) ระหว่างตัวยับยั้งกับโครงสร้างเอนไซม์อินติเกรสสมบูรณ์นับเป็นอุปสรรคสำคัญต่อกระบวนการพัฒนายายับยั้งเอนไซม์อินติเกรส ดังนั้นใน

งานวิจัยนี้จึงได้ทำการสร้างโครงสร้างสมบูรณ์ของเอนไซม์อินติเกรสจากโครงสร้างทางการทดลองที่ประกอบด้วย 2 ส่วน จำนวน 2 โครงสร้าง ด้วยวิธีการทางเคมีคอมพิวเตอร์ และศึกษาแรงกระทำระหว่างเอนไซม์อินติเกรสกับตัวยับยั้งด้วยวิธีโมเลคิวลาร์ไดนามิกส์ซิมุเลชัน

ผลการวิจัย สามารถสร้างโครงสร้างสมบูรณ์ของอินติเกรสได้สำเร็จจากโครงสร้าง 2 ส่วนของอินติเกรสจำนวน 2 โครงสร้าง จากนั้นทำการคำนวณจำลองระบบด้วยวิธีการโมเลคิวลาร์ไดนามิกส์ซิมุเลชันของระบบ core อิสระ กับระบบโครงสร้างสมบูรณ์อิสระ (ไม่มีตัวยับยั้ง) ผลการวิเคราะห์เปรียบเทียบสมบัติพลวัติต่าง ๆ เช่น โครงสร้าง ความยืดหยุ่นของกรดอะมิโนสำคัญในบริเวณเร่งระหว่าง 2 ระบบนี้ พบว่า N- และ C-terminal มีอิทธิพลอย่างมากต่อโครงสร้างส่วน core ข้อมูลนี้บ่งชี้ว่าควรใช้โครงสร้างสมบูรณ์สำหรับการศึกษาโครงสร้างการเข้าจับระหว่างเอนไซม์อินติเกรสกับตัวยับยั้ง และจากการคำนวณจำลองระบบของระบบโครงสร้างสมบูรณ์ที่จับกับตัวยับยั้ง และระบบ Core เดี่ยว ๆ จับกับตัวยับยั้งยืนยันว่า N- และ C-terminal มีอิทธิพลต่อสมบัติพลวัติต่าง ๆ ของโครงสร้างส่วน core ที่เป็นบริเวณการเข้าจับกับตัวยับยั้ง ผลที่ได้นี้จะมีประโยชน์ต่อกระบวนการพัฒนายารักษาโรคเอดส์ชนิดยับยั้งการทำงานของเอนไซม์อินติเกรส

ภาคผนวกที่ 4

4. กิจกรรมอื่น ๆ ที่เกี่ยวข้อง

4.1 การนำเสนอผลงานทางวิชาการในการประชุมทางวิชาการ

- เสนอผลงานวิจัยในรูปแบบโปสเตอร์ หัวข้อเรื่อง HIV-1 integrase: Structure and Dynamic as Studied by Molecular Modeling and Molecular Dynamics Simulation ในงานประชุม 48th Biophysical Society Annual Meeting ระหว่างวันที่ 14-18 กุมภาพันธ์ 2547 ณ เมือง Baltimore รัฐ Maryland ประเทศสหรัฐอเมริกา
- เสนอผลงานวิจัยในรูปแบบโปสเตอร์ หัวข้อเรื่อง Molecular Modeling and Molecular Dynamics Simulations of Full-length HIV-1 Integrase ในงานประชุม 20th Austin Symposium on Molecular Structure ระหว่างวันที่ 7-9 มีนาคม 2547 ณ เมือง Austin รัฐ Texas ประเทศสหรัฐอเมริกา
- เสนอผลงานวิจัยในรูปแบบโปสเตอร์ หัวข้อเรื่อง A Full-length HIV-1 Integrase: Molecular Modeling and Molecular Dynamics Simulations ในงานประชุม 8th Annual International Conference on Research in Computational Molecular Biology ระหว่างวันที่ 27-31 มีนาคม 2547 ณ เมือง San Diego รัฐ California ประเทศสหรัฐอเมริกา
- เสนอผลงานวิจัยในการประชุมประจำปี เมธีวิจัยอาวุโส สำนักงานกองทุนสนับสนุนการวิจัย ของ รองศาสตราจารย์ ดร. สุพจน์ หารหนองบัว เรื่อง การออกแบบเชิงโมเลกุลของวัสดุและยาโดยวิธีทางเคมีคอมพิวเตอร์ ในวันที่ 14 สิงหาคม 2547 ณ ห้องจามจุรี 1 โรงแรมปทุมวันปริ๊นเซส ในหัวข้อเรื่อง โครงสร้างสามมิติที่สมบูรณ์ของ HIV-1 Integrase จากการศึกษาโดยวิธีโมเลคิวลาร์ไดนามิกส์ซิมุเลชัน