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The conformational feasibility for the formation of reaching dimer in ASV and HIV Integrase: a molecular dynamics study

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Abstract

Retroviral integrases are reported to form alternate dimer assemblies like the core-core dimer and reaching dimer. The core-core dimer is stabilized predominantly by an extensive interface between two catalytic core domains. The reaching dimer is stabilized by N-terminal domains (NTD) that reach to form intermolecular interfaces with the other subunit's core and C-terminal domains (CTD), as well as CTD-CTD interactions. In this study, molecular dynamics (MD), Brownian dynamics (BD) simulations, and free energy analyses, were performed to elucidate determinants for the stability of the reaching dimer forms of full-length ASV and HIV IN, and to examine the role of the C-tails (the last ~16–18 residues at the C-termini) in their structural dynamics. The dynamics of an HIV reaching dimer derived from SAXS and protein crosslinking data, was compared with the dynamics of a core-core dimer model derived from combining the crystal structures of two-domain fragments. The results showed that the core domains in the ASV reaching dimer express free dynamics, whereas those in the HIV reaching dimer are highly stable. Brownian dynamics simulations suggest a higher rate of association for the HIV core-core dimer than the reaching dimer. The predicted stability of these dimers was therefore ranked in the following order: ASV reaching dimer < HIV reaching dimer < composite core-core dimer. Analyses of MD trajectories have suggested residues that are critical for intermolecular contacts in each reaching dimer. Tests of these predictions and insights gained from these analyses could reveal a potential pathway for the association and dissociation of full-length IN multimers.

Keywords

ASV; HIV; integrase; reaching dimer; core-core dimer

1. Introduction

Retroviral integrase catalyzes the insertion of viral DNA into the host DNA and hence is an indispensable enzyme for the survival of retroviruses (Fujiwara & Craigie 1989; Bushman &

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Supplementary Material

This article contains Supplementary Figures 1-3 and Supplementary Tables 1-10.

Craigie 1990; Bushman, Fujiwara & Craigie 1990; Katz, Merkel, Kulkosky, Leis & Skalka 1990; Goff 1992; Pommier, Johnson & Marchand 2005; Adamson & Freed 2010; Moreno et al. 2010). Integrase (IN), a 32 kDa protein in Human Immunodeficiency Virus (HIV), is a promising anti-HIV target and several classes of inhibitors have been reported (Gupta, Roy & Garg 2009; Plewe et al. 2009; Cheng, Zhang & Fu 2010; Tanis et al. 2010; Johnson et al. 2011; Sharma et al. 2011; Telvekar & Patel 2011). IN active-site-directed strand transfer inhibitors, Raltegravir, Elvitegravir and Dolutegravir have been approved by Food and Drug Administration for anti-retroviral therapy (<http://aidsinfo.nih.gov/guidelines>; Summa et al. 2008; Volberding & Deeks 2010). These drugs are recommended for HIV infected patients with resistance against reverse transcriptase and protease inhibitory drugs. However, the emergence of resistant mutations against all the available anti-retroviral drugs has necessitated the design and development of new drugs and strategies to inhibit HIV (Al-Mawsawi, Al-Safi & Neamati 2008; Cooper et al. 2008; Ceccherini-Silberstein et al. 2009; Serrao, Odde, Ramkumar & Neamati 2009). A major set-back in studying HIV IN as a target is the lack of full-length crystallographic or NMR structures due to the flexible inter-domain linkers and its tendency to form higher order aggregates (Cheng et al. 2007; Neamati 2011).

IN catalyzes the viral and host DNA integration via a two-step reaction (3'-processing and DNA joining or strand transfer) in the presence of two Mg^{2+} ions (Sherman & Fyfe 1990; Bushman & Craigie 1991; Engelman, Mizuuchi & Craigie 1991). The three domains of IN (Engelman & Craigie 1992) namely N-terminal domain (NTD) (Cai et al. 1997), catalytic core domain (CCD) (Engelman, Bushman & Craigie 1993) and C-terminal domain (CTD) (Lodi et al. 1995), connected by flexible linkers participate in multimerization, catalytic and DNA binding activities. Several crystallographic or NMR solution structures of isolated HIV-1 IN domains are deposited in Protein Data Bank, which include 6, 15 and 2 structures for NTD, CCD and CTD, respectively. The PDB IDs of the structures of these isolated domains are; (i) NTD: 1WJA-1WJF (ii) CCD: 1ITG, 2ITG, 1BHL, 1BI4, 1BL3, 1BIS, 1BIU, 1BIZ, 1B92, 1B9D, 1B9F, 1QS4, 1EXQ, 1HYV and 1HYZ and (iii) CTD: 1IHV and 1QMC (Jaskolski, Alexandratos, Bujacz & Wlodawer 2009). Additionally the two-domain constructs such as NTD-CCD (PDB ID: 1K6Y) and CCD-CTD (PDB ID: 1EX4) are reported by Wang et al and Chen et al, respectively (Wang, Ling, Yang & Craigie 2001, Chen et al. 2000). But these studies fail to show the positions of domains in the full-length IN and the last 18 residues at the C- terminus (C-tail). IN is functional in dimeric or tetrameric forms and all three domains of IN exist as dimers in isolated states (Ellison, Gerton, Vincent & Brown 1995; Bao et al. 2003; Guiot et al. 2006; Jaskolski et al. 2009). An IN dimer could catalyze both 3'-processing and strand transfer reactions for a single viral DNA end integration, whereas, a tetramer is the smallest multimer required for concerted integration of both the viral DNA ends into host DNA (Jones, Coleman, Merkel, Laue & Skalka 1992; Li & Craigie 2005; Li, Mizuuchi, Burke & Craigie 2006).

The highly polymorphic nature of IN and its multimers provide an additional complexity in designing potent inhibitors against IN. Hence, identifying and characterizing the structure of full-length IN in its functional state is an important preliminary step towards targeting IN. In this context, several models of full-length HIV-1 IN have been constructed based on the available experimental data (Heuer & Brown 1998; Podtelezhnikov, Gao, Bushman & McCammon 2003; Wielens, Crosby & Chalmers 2005). In later models the NTD-CCD and

CCD-CTD two-domain structures of IN were used primarily to generate full-length structures in monomeric and dimeric forms, while only few attempts were successful in deriving a functional tetrameric state (Wang et al. 2001; Balasubramanian, Rajagopalan & Ramaswamy 2012). The rotational correlation times and spatial arrangements of various IN domains have been elucidated from the available crystallographic and experimental data (Podtelezhnikov et al. 2003).

The precise information on the spatial arrangement of IN domains in a functional intasome (viral DNA bound tetramer) was not clear until the structure of prototype foamy virus (PFV) intasome was solved by Hare et al, in 2010 (Hare, Gupta, Valkov, Engelman & Cherepanov 2010). In addition to DNA binding, the inner monomeric subunits of PFV intasome are associated through the interactions between NTD of one monomer and CCD of another monomer, while the two outer monomeric units interact with the inner monomers by CCD-CTD interactions. The cryo-EM and X-ray studies of Mouse mammary tumour virus (MMTV) and Rous sarcoma virus (RSV) intasomes have reported an octameric state of IN bound to both viral and target DNA (Ballandras-Colas et al. 2016; Yin et al. 2016). Similar to the PFV intasome, the inner dimers of both MMTV and RSV express domain-swapping interactions, while the outer dimers provide CTDs to the inner dimers for integration in a *trans* fashion. Such difference in the multimeric states, required for IN function, is due to the varying length of NTD-CTD as well as CCD-CTD linkers. The longer CCD-CTD linker in PFV facilitates tetramer formation, while the shorter CCD-CTD linker in MMTV and RSV requires an octameric state. The distal IN dimers of MMTV and RSV having the alternate CTD positioning provide the key CTD, that engages viral DNA analogous, to the inner CTDs of the PFV intasome. These studies highlight the importance of the length of linker region and its flexibility in determining the multimeric states required for a functional intasome formation among viruses.

Small angle x-ray scattering (SAXS) and biochemical cross-linking analyses were used by Bojja et al, to elucidate the structure of avian sarcoma virus (ASV) (Bojja et al. 2011) and HIV IN (Bojja et al. 2013) in the absence of viral DNA (apo-form). These structures possess a dimer interface similar to the inner monomers of PFV intasome. The NTDs and CTDs form the dimer interface of ASV and HIV IN, where NTD of one monomer interacts with both CCD and CTD of another monomer, while the CTDs of both monomers interact with each other. Such dimer conformation was named a “reaching dimer,” which is in contrast to the CCD-CTD dimer interface (termed composite dimer or core-core dimer) reported in the available crystal structures (Goldgur et al. 1998; Goldgur et al. 1999; Chen et al. 2000; Wang et al. 2001). The plasticity of inter-domain linkers allows variable spatial arrangements of IN domains in the presence and absence of DNA (Alian et al. 2009), while the physiological and functional relevance of the resulting interfaces (reaching and core dimer interfaces) remain yet to be fully understood. Following the ASV reaching dimer, a full-length reaching dimer of HIV-1 IN has also been reported in apo-form (Bojja et al. 2013), which shows a modified spatial arrangement of the IN domains when compared with ASV IN. The difference in the spatial arrangements between the ASV and HIV dimer models reflect the plasticity of IN linkers and the hydrophobicity of the dimer interfaces (Bojja et al. 2013).

Computational methods such as molecular modeling and molecular dynamics (MD) simulations have gained importance in identifying biomolecular structures and also in understanding their structural as well as functional dynamics (Costantino, Entrena-Guadix, Macchiarulo, Gioiello & Pellicciari 2005; Villa, Wohner & Stock 2009; Huggins et al. 2010; Casoni, Clerici & Contini 2013; He et al. 2013). In recent years, molecular dynamics studies have been used to explore the structural dynamics of HIV proteins including protease, reverse transcriptase and IN (Lins et al. 1999; Madrid, Jacobo-Molina, Ding & Arnold 1999; Madrid, Lukin, Madura, Ding & Arnold 2001; Hornak, Okur, Rizzo & Simmerling 2006; Liu, Liu & Fu 2008; Kim, Hartley, Curran & Engelman 2009; Yokoyama, Naganawa, Yoshimura, Matsushita & Sato 2012). Several studies have elucidated the mechanism of DNA processing and inhibition of HIV IN using its tetrameric form model based on the PFV intasome (Krishnan et al. 2010; Xue, Liu & Yao 2011; Xue et al. 2012). In addition, structural and energetic insights into the complex formation pathway of several multimeric proteins have been elucidated successfully by molecular dynamics simulations (Gilson & Zhou 2007; Stoica, Sadiq & Coveney 2008; Hou, Wang, Li & Wang 2010; Hu, Wang, Liu & Zhang 2010; Rastelli, Del Rio, Degliesposti & Sgobba 2010; Hu, Zhang & Chen 2011; Sangeetha, Muthukumaran & Amutha 2015). In recent years, protein-protein interaction kinetics and the elucidation of association/dissociation rates have been investigated by computational methods, due to various limitations in experimental procedures such as the limited accessible rates (difficult for reactions faster than $\sim 10^6 \text{ M}^{-1}\text{s}^{-1}$) and occurrence of multiple short living transition states (Gabdoulline & Wade 1997; Spaar, Flock & Helms 2009; Elsayy, Caves & Twarock 2010; ElSawy et al. 2013). Brownian dynamics (BD) simulations are used widely to calculate the rate of protein-protein associations and a reaction is considered as a fast reaction, when the rate of association is greater than $10^6 \text{ M}^{-1}\text{s}^{-1}$. Protein multimerization depends on both long-range and short-range interactions, which diffuse the proteins or lead to multimerization, respectively, via the formation of an intermediate (also called the encounter complex). The encounter complex is formed as a result of the conformational changes required for multimerization and hence the rate of formation of encounter complex is indicative of the association rate (DeLisi 1980). The association rates of several protein complexes including barnase-barstar and MS2 viral capsid have been elucidated by brownian dynamics and are well in agreement with the experimental rates (Gabdoulline & Wade 1997; Spaar, Dammer, Gabdoulline, Wade & Helms 2006; Elsayy et al. 2010).

The present study explores the stability of the dimers of full-length ASV and HIV IN along with the role of C-tail in their structural dynamics. Also, a model of HIV reaching dimer based on ASV was generated to understand the structural preference between ASV and HIV reaching dimers. The difference in the structural stability among these three conformations, and the role of C-tail on their structural dynamics are explained. The structural dynamics of HIV reaching dimer was compared with that of the core dimer and their association rates are also reported. Altogether, the insights gained from these analyses could reveal a potential pathway for the association and dissociation of full-length IN multimers.

2. Materials and Methods

2.1. Molecular dynamics simulation

The C-tails of both ASV and HIV IN structures were modeled in an extended conformation (i.e. in a linear fashion without any secondary structures) and without interaction with the rest of the structure. The protocol adopted in our previous study (Balasubramanian et al. 2012) was used in modeling the C-tails. The structures were simulated for a period of 5 ns by fixing all the domains and linkers except the modeled C-tails. The conformations resulted from the 5 ns MD trajectory were subjected to clustering analysis by average linkage algorithm to identify a representative starting structure, which was used for further unrestrained simulations for a period of 50 ns. The co-ordination of Zn^{2+} ion by the NTD is important for its stability and multimerization. Since all the reaching dimer conformations possessed the E-form of N-terminal domain, the HHCC motif and Zn^{2+} ion was modeled in E-form co-ordination (Sangeetha, Muthukumaran & Amutha 2014). Previous simulations of full-length IN in monomeric and dimeric forms in the absence of Zn^{2+} ion revealed an unstable NTD where the helical bundle does not hold properly. Hence, the Zn^{2+} coordination was included in all simulations in this study. MD simulations were performed using AMBER11 and parm99 (ff99SB) protein force field (Hornak et al. 2006; Case et al. 2010). TIP3P (Jorgensen, Chandrasekhar, Madura & Klein 1983) water model was used to solvate the IN structures with water extending up to 10 Å from all sides of the protein. The solvated systems were energy minimized by steepest descent followed by conjugate gradient methods. The long-range interactions were treated using particle-mesh Ewald procedure and were truncated at a cut-off of 10 Å. The minimized systems were heated to 300 K in the NVT ensemble over a period of 50 ps. The density of the heated simulation system was adjusted to 1 atm in the NPT ensemble and relaxed over a period of 500 ps. The systems were subjected to unrestrained production simulation for a period of 50 ns. The conformations sampled over the entire 50 ns trajectory were clustered based on distance-RMSD with a minimum cut-off distance of 4 Å between clusters. All the analyses were performed using the cpptraj module of AmberTools 13. In order to distinguish the IN residues of both chains, the residues of chain “B” are referred with an apostrophe. The Radius of gyration (R_g) of the dimer interfaces were calculated by considering the entire NTD and CTD domains. The residues 1-54, 1-55, and 1-57 were considered as NTD, while 214-368, 201-269 and 217-269 were considered as CTD for ASV, HIV_A and HIV reaching dimers, respectively. The difference in domain boundaries considered for R_g calculation of HIV_A and HIV reaching dimers is due to the variation in the three-dimensional arrangement among the reaching dimers.

2.2. MM/GBSA Binding energy analysis

The binding energy of IN dimers was analyzed by MM/GBSA method implemented in AMBER11 (Miller et al. 2012). Snapshots at 100 ps interval were extracted from the last 10 ns of the simulation and the binding free energy was computed as an average of these snapshots using the expression given below.

$$\Delta G_{bind} = \Delta G_{dimer} - (\Delta G_{monomerA} + \Delta G_{monomerB})$$

Where,

$$\Delta G = \Delta G_{gas} + \Delta G_{sol}$$

$$\begin{aligned}\Delta G_{gas} &= \Delta E_{bond} + \Delta E_{angle} + \Delta E_{dihedral} + \Delta E_{ele} + \Delta E_{vdW} \\ \Delta G_{sol} &= \Delta E_{pol} + \Delta E_{nonpol} \\ \Delta E_{nonpol} &= \gamma SASA + \beta\end{aligned}$$

The terms G_{dimer} , $G_{monomerA}$, and $G_{monomerB}$ represent the average free energies of the dimer, monomer A and monomer B respectively. G_{gas} , the gas phase (vacuum) molecular mechanics energy is calculated as the sum of internal (bond E_{bond} , angle E_{angle} , dihedral $E_{dihedral}$), electrostatic (E_{ele}) and vdW (E_{vdW}) energies. The solvation energy, G_{sol} includes polar G_{pol} and non-polar G_{nonpol} terms. The polar component is calculated using Generalized Born approximation with a grid spacing of 0.5 Å and the dielectric constants, 1.0 and 80.0 for solute and solvent, respectively. The non-polar component is obtained by calculating the solvent accessible surface area using a probe of radius 1.4 Å and Linear Combination of Pairwise Overlaps (LCPO) approximation (Weiser, Shenkin & Still 1999).

2.3. Brownian Dynamics Simulation

The BD simulations were performed using the SDA 7.0 package (Gabdouline & Wade 1997; Gabdouline & Wade 1998) by considering the proteins as rigid entities with translation and rotational diffusion properties. The propagation of BD simulations was achieved by solving the translational and rotational diffusion equations using the Ernak-McCammon algorithm (Ermak & McCammon 1978). The translational and rotational diffusion coefficients of INs were calculated using the HYDROPRO software (Garcia De La Torre, Huertas & Carrasco 2000). The two IN monomers were placed at an initial separation (b) of 150 Å from the centre of masses (COM) and the BD trajectories were terminated, when the COM-COM distance exceeds three times the initial separation ($c = 3b$). The major driving forces included in this BD simulation are: steric, desolvation and electrostatic interactions. Steric interactions were implicitly accounted by using exclusion grids with grid spacing of 1 Å to prevent overlaps. The electrostatic potential around the proteins were calculated using APBS program (Baker, Sept, Joseph, Holst & McCammon 2001) with a grid dimension of $161 \times 161 \times 161$ Å and 1 Å grid spacing. A dielectric of 78 is used to define the solvent and the protein interior dielectric constant was set to 2 at a salt concentration of 0.5 M. The electrostatic desolvation was empirically obtained using the desolvation grid module (Elcock, Gabdouline, Wade & McCammon 1999) of SDA package. The atomic charges and radii for all the protein atoms were taken from the AMBER ff99SB force field and the effective charges were derived from the ECM module (Gabdouline & Wade 1996) of SDA package. The formation of an encounter complex is considered as a “reaction” and the hydrogen bonding pairs at the dimer interfaces were used as the reaction criteria. In this study, a “reaction” is said to occur, when two or three contacts (hydrogen bonding pairs) are formed simultaneously and are monitored within 0 and 20 Å separation in steps of 0.5 Å. Independency among the resulting contacts was ensured by defining a contact as a reaction event, only if the contact atoms in the same monomer are beyond 4.5 Å. About

100,000 trajectories for each protein complex were simulated with random orientations of two proteins at distances b and truncated when the protein COMs exceed c . Among these trajectories, if a fraction satisfies the reaction criteria, the rate constant $k(x)$ at a relative separation of x can be calculated by combining the probability of occurrence of any of the reaction events (β) and translational diffusion coefficient D ($k(b) = 4\pi Db$) (Northrup, Allison & McCammon 1984) of the proteins using the relationship shown below,

$$k(x) = \frac{k(b)\beta}{1 - (1-\beta)k(b)/k(c)}$$

The rate constant calculated for these dimers were used to explain the ease of complex formation.

3. Results and Discussion

The reaching dimer conformation of IN is an extended dimeric form characterized by interactions between NTD and CTD of one monomer with both NTD and CTD of another monomer along with CTD-CTD' interactions. While at a different angle, the CTD-CTD' interface of IN reaching dimer is similar to that observed in isolated CTD dimer NMR structures (Eijkelenboom et al. 1995; Lodi et al. 1995; Eijkelenboom et al. 1999) and is stabilized by hydrophobic interactions along with a π -stacking interaction between Trp243 (Trp259 in ASV) of both monomers. Molecular dynamics simulations over a period of 50 ns were performed for a SAXS-derived ASV reaching dimer, an ASV based HIV model (reference, hereafter called HIV_A) and a SAXS-derived HIV IN reaching dimer (Bojja et al. 2013) and their structural stabilities analyzed in this study. The influence of C-tail (residues 269-286 in ASV and 270-288 in HIV) on the structure and dynamics of full-length INs was also analyzed by simulating the reaching dimers in the presence and in the absence of C-tail.

3.1 Dynamics of ASV IN reaching dimer

The RMSD value, which explains the variation in the position of the backbone atoms of IN during the simulation with respect to the initial conformation, was calculated for ASV IN in the presence and absence of C-tail (hereafter referred as ASV-tail and ASV-notail, respectively) and is shown in Figure 1(a). Both these structures show a highly dynamic nature and required a longer time period to equilibrate (~ 22 ns). Results from the initial phase of the simulation indicate that ASV IN is more stable in the presence of C-tail than in its absence. Whereas, after 25 ns simulation, the RMSD of ASV-tail increases considerably. The average positional fluctuations of C α -atoms (Figure 1(b)) among the monomeric chains of both ASV-tail and ASV-notail show a similar pattern. In ASV-tail, both the C-tails and one of the CTDs show increased fluctuations, while the rest of the structure is stable when compared to ASV-notail. The R_g (Figure 1(c)) of both ASV-tail and ASV-notail structures are similar (falling in the range of 33–36 Å) which is also consistent with the experimentally determined R_g value of 33.1 ± 0.6 Å (Bojja et al. 2011). On the other hand, R_g of the dimer interface (Figure 1(d)) comprising the NTD and CTD stabilizes only after 20 ns. After 35 ns simulation, the dimer interface R_g of ASV-tail decreases while that of ASV-notail increases.

The π -stacking interaction (Figure 2) between Trp259 of the two CTDs is weak (~ 6 Å) in the presence of C-tail while it is completely absent (> 7 Å) in ASV-notail. After 35 ns, when the Rg of ASV-tail dimer interface decreases, the Trp259 residues move apart up to 12 Å and hence the weak π -stacking interaction becomes completely absent. The C α atoms of residues Lys34 (in $\alpha 3$ of NTD), Val75 (in $\beta 2$ of CCD), and Val 250 (in $\beta 3$ of CTD) were used as references to calculate the inter-domain distances and the variation during the simulation is shown in Supplementary Figure 1. Among the calculated inter-domain distances, only NTD-CTD distance is relatively stable. Due to the increased flexibility of the inter-domain linkers, the CCD shows high degree of fluctuations and hence its distance with NTD and CTD varies considerably both in the presence and absence of C-tail.

The conformations sampled throughout the 50 ns simulation were clustered to identify the representative conformation for both ASV-tail and notail, which resulted in 5 and 7 clusters, respectively. Structural superimposition of the initial and representative conformation from the most populated cluster for both ASV-tail and notail simulations is shown in Figure 3(a), where the increased conformational dynamics of CCD is clearly depicted. In both the full-length ASV IN structures, a spatial displacement of NTD is observed while one of the CCDs expresses a dynamic nature with respect to the NTD-CCD linker. Lack of interacting partners, flexible inter-domain linkers and intra-monomer H-bonds between flexible C-tail and CCD might be responsible for this free dynamics of the CCD. The surface area and volume of the dimer interface before and after 50 ns simulation for the initial and the 50 ns MD simulated conformation is calculated in Table 1. It is observed that in the presence of C-tail the buried surface area remains the same while the volume of the dimer interface alone increases after 50 ns simulation. Contrastingly, both the buried surface area and volume increase considerably in the absence of C-tail and attains a value similar to ASV-tail. These observations imply an important role for C-tail in stabilizing the ASV reaching dimer interface.

3.1.1. H-bonding network—H-bond interactions that are stable during more than 10 % of the MD trajectory were analyzed and reported in this study. The C-tail expresses several intra-monomer H-bonds with both the CCD and CTD while forming only one inter-monomer H-bond with Asn24 (NTD) (Supplementary Table 1). The dynamic nature of C-tail is responsible for the difference in the previously reported potential stabilizing interactions and theoretically identified interactions. However, when considering the entire reaching dimer, numerous H-bonds are observed to stabilize this dimer interface (Figure 4(a)) and are reported in Supplementary Tables 2 and 3. One of these, H-bond between Glu32 and Arg263' is consistent with the reported prediction of potential stabilizing interactions in the ASV reaching dimer (Bojja et al. 2011), and is found to be stable during the conformational evolution of ASV-tail. Apart from this, two H-bonds, Arg214 – Met27' and Leu218 – Gln28' were observed between residues close to the reported potential H-bond Gln28 – Thr216. Additionally, inter-monomer H-bonds such as Gln28 – Gly243', Trp213 – Arg17', Asp255 – Arg263'; and Glu256 – Lys264' were identified to stabilize the dimer interface of ASV-tail, some with prominent lifetimes during the 50 ns simulation.

ASV-notail expresses an H-bond interaction between Gln28' and Ser262, in addition to a mutual H-bond between Glu32 and Arg263. Gln28' also forms H-bonds with several

residues such as Gly243, Gly245 and Tyr246 at various periods during the simulation. H-bonds between residues Lys21, Asn24, Gln28 and Leu218 of one monomer and His212', Lys211', Val265', and Met27' of another monomer, respectively were observed, which are close to the reported potential H-bonding pairs (Bojja et al. 2011) Ser20-Trp213, Asn24-Arg214, Gln28-Ser262, Asn24-Arg53 and Gln28-Thr216. A consistent H-bond between Arg31 and Glu220' is observed instead of the reported potential H-bond between Arg31 and Arg244. Glu220 is a part of the CCD-CTD linker while Arg244 is present in CTD- β 3. The shift in H-bond from CTD to CCD-CTD linker clearly reflects disturbances in the dimer interface. It was reported that the RGY (residues 244-246) loop could form inter-monomer H-bonds with its counterpart and NTD (Bojja et al. 2011). Few such interactions are observed both in the presence and absence of C-tail. The remaining H-bonds involving NTD, CTD and CCD-CTD linker are transient, reflecting the dynamic nature of the dimer interface.

3.2. Dynamics of HIV IN reaching dimer based on ASV IN

The reaching dimer model of ASV IN was used to generate a full-length reaching dimer model for HIV IN. ASV and HIV IN possess less than 20 % sequence similarity yet the topology of the individual domains and their mechanism of action are similar (Katz et al. 1990; Bushman & Wang 1994; Jaskolski et al. 2009). The stability of this modeled HIV_A reaching dimer and the influence of C-tail (residues 270-288) on its dynamics was analyzed by molecular dynamics simulations in the presence and in the absence of C-tail.

The RMSD of HIV_A IN shows that the structure is stable in the presence of C-tail (Figure 1(a)). In the absence of C-tail, the HIV_A IN reaching dimer fails to stabilize even after 20 ns MD simulation and accordingly the RMSD increases from 4 to 16 Å by the end of the simulation. This instability is also reinforced by the increased RMS fluctuations of Ca atoms from the mean position in the absence of C-tail (Figure 1(b), compare blue to green traces). The C-tails show increased fluctuation and expresses both intra-monomer and inter-monomer H-bonds (Supplementary Table 4). Since HIV_A IN model was built based on the ASV reaching dimer, the Rg of HIV_A IN (34 ± 1 Å) is also similar to ASV MD simulations (Figure 1(c)) and closer to its experimentally determined ASV reaching dimer Rg value of 33.1 ± 0.6 Å (Bojja et al. 2011). The Rg of dimer interface (Figure 1(d)) is stable both in the presence and absence of C-tail. In the absence of C-tail, the CTD interface and Trp243 π -stacking interaction are highly stable within the range of 5.22 ± 0.5 Å (Figure 2). Similar to the variations observed in the inter-domain distances of ASV IN during MD, the HIV_A IN CCD shows increased fluctuation (Supplementary Figure 1) and hence its distance from both NTD and CTD varies considerably irrespective of the presence or absence of C-tail.

The clustering of all sampled conformations from the 50 ns simulation trajectory resulted in 4 and 5 clusters in the presence and absence of C-tail, respectively. The dynamic nature of both INs in the presence and absence of C-tail is clearly visible from superimposition of the initial and most populated cluster (Figure 3(b)). The small α -helix formed by the residues (Ile217 to Asn222) of CCD-CTD linker is stable in the presence of C-tail, while it becomes a coil in the absence of C-tail (Figure 4(b)) causing more flexibility and hence results in a highly dynamic CCD. Since the residues Ile217 to Asn222 form an α -helix, the CTD β -

barrel is not formed completely in the modeled initial structure. Accordingly, the $\beta 1$ and $\beta 5$ sheets are not formed even after the simulation of 50 ns, but are present as an extended coil irrespective of the presence or absence of C-tail. The surface area and volume of the initial and final most populated cluster from the MD simulation was calculated (Table 1) to observe the stability of the dimer interface. The buried surface area decreases considerably in the presence of C-tail while there is no major deviation in the absence of C-tail. The volume of dimer interface varies both in the presence and absence of C-tail and attains $\sim 5800 \text{ \AA}^3$ after 50 ns MD simulation in the absence of the tail.

3.2.1. H-bonding network—The intra- and inter-monomeric H-bond interactions expressed by C-tail are extracted and shown in Supplementary Table 4. Among these interactions, the H-bond between Asp270 and Arg231 is observed to be consistent and mutual between the two monomers. In addition to these H-bonds, the dimer interface is stabilized by H-bonds between residues of NTD and CTD of both the monomers (Figure 4(b), Supplementary Tables 5 and 6). The reported potential H-bonds (Bojja et al. 2011) are absent in HIV_A reaching dimer in the presence of C-tail. A mutual H-bond between Asp6 and Arg269 is observed among the two monomers. An H-bond is formed between Glu35 (NTD) of one monomer and Arg262' (CTD) of another monomer, which is closer to the reported potential H-bond between Glu35 and Arg263. In contrast to this, one of the dimer interfaces expresses Glu35'-Lys216 H-bond interaction signifying a disturbance in the orientation of NTD and the dimer interface.

In the absence of C-tail, a pair of mutual H-bonds (Glu35-Arg262' and Ser39-Arg263') were identified which are close to the reported potential H-bonds Arg263 - Glu35 and Arg262-Pro30, Val31. Apart from these, H-bonds like Asp6-Arg269' (mutual), Glu10-Arg269', and Glu35-Arg269' were identified to stabilize the dimer interface. Similar to the case in the presence of C-tail, the Glu35'-Lys215 H-bond is observed in one of the dimer interfaces reflecting a clear disturbance in the dimer interface. The formation of a new H-bond interaction between Glu13 of NTD $\alpha 1$ helix (which faces opposite to the dimer interface initially) and Arg263 (3_{10} helix of CTD) implies a rotation of NTD and thereby disturbing the dimer interface. These alternate interactions highlight the potential variability of reaching dimer interactions in this model of the HIV dimer based on the analogous ASV dimer.

3.3. Dynamics of HIV IN reaching dimer

As documented in the subsequent study with SAXS and cross-linking methods, HIV IN attains a reaching dimer conformation that is different from the ASV IN conformation (Bojja et al. 2013). Though the length of ASV and HIV protein sequence is virtually the same (286 in ASV and 288 in HIV), their sequence similarity is less than 20 % and hence the secondary structural propensities may also vary. The difference in the arrangement of various domains among HIV and ASV IN is due to the inter-domain linkers. A short α -helix is reported to form in the CCD-CTD linker region (residues 203-222) of HIV (Jaskolski et al. 2009). In agreement with this, both HIV and HIV_A reaching dimer express a short α -helix by the residues 210-217 and 217-222, respectively. Though the HIV_A reaching dimer possesses an α -helix, it is located closer to the CTD and hence, the $\beta 1$ and $\beta 5$ sheets are not

formed in this conformation. On the other hand, in the later SAXS identified HIV reaching dimer, this α -helix begins from the residue Thr210. A kink in the CCD-CTD linker originating at Thr210 is previously reported to facilitate *cis* and *trans* activity of CTD resulting in asymmetric and functional IN. (Chen et al. 2000; Balasubramanian et al. 2012) Also in the HIV reaching dimer, the topology of CTD β -barrel is intact. The secondary structural propensities calculated for the inter-domain linker residues also reinforce the secondary structures observed in HIV reaching dimer (Supplementary Figure 2). The α 1 of NTD and C-tail are predicted to be disordered and potentially involved in protein-protein interactions by DISOPRED and ANCHOR analysis (Jones & Cozzetto 2014) of both ASV and HIV (Supplementary Figure 3). In addition, the CCD-CTD linker of HIV is also predicted to be disordered and involve in protein-protein interactions. The structure of HIV reaching dimer is evidently in accordance with the secondary structural propensities of its sequence and hence its stability as well as dynamics was analyzed by molecular dynamics simulations.

The RMSD of HIV reaching dimer in the presence and absence of C-tail stabilizes considerably faster (~ 10 ns) than the other simulated structures (Figure 1(a)). In contrast to the previous simulations, the dimer in the presence of C-tail expresses a slightly higher RMSD than in the absence of C-tail by ~ 2 Å, while both display overall RMSDs below all of the other simulations described above. All three domains in both the structures show stable dynamics as seen by their RMS fluctuations, where the C-tail alone expresses a dynamic nature (Figure 1(b)). The radius of gyration of both the structures is stable with the dimer in the presence of C-tail expressing ~ 2 Å higher R_g than in the absence of C-tail (Figure 1(c)). The average R_g of both the dimer interfaces (Figure 1(d)) identified by MD simulations is ~ 33 Å while the experimentally calculated R_g is 40 Å (Bojja et al. 2013). Though, the R_g of complete protein show small fluctuations, the R_g of their dimer interfaces are highly stable. In spite of a stable dimer interface, both these structures fail to express the π -stacking interaction between Trp243 of the two CTDs (Figure 2). The inter-domain distances between the NTD, CCD and CTD were calculated for both the monomers and plotted in Supplementary Figure 1. From this figure, it is clear that, the HIV reaching dimer expresses least variation in the inter-domain distances among all the simulated IN structures and hence is highly stable.

The HIV reaching dimer in the presence and absence of C-tail resulted in 4 and 2 clusters, respectively and their structural superimposition with the initial conformation is shown in Figure 3(c). The structures do not show major spatial deviations as observed in the HIV_A IN models. The surface area and volume of the dimer interface before and after 50 ns simulation in the presence and absence of C-tail were calculated and given in Table 1. The volume of the dimer interface shows a considerable increase in both the dimer structures, while the buried surface area remains stable.

3.3.1. H-bonding network—The C-tail of both monomers expresses a number of intra-monomeric H-bonds with the NTD and CCD while no inter-monomeric H-bonds are formed (Supplementary Table 7). In the presence of C-tail, the dimer interface is majorly stabilized by NTD-NTD (10 H-bonds) interactions and 7 H-bonds between the CTD and NTD. Also, the CTD-CTD interface is stabilized by the presence of four H-bonds (Figure 4(c)). A few

H-bonds at the NTD-NTD and CTD-CTD interface are seen both in the presence and absence of the C-tail. For example, the CTD-CTD interface H-bond between Asp256 and Ser230 is present in both simulations with significant lifetimes. All H-bonds stabilizing the dimer interface are reported in Supplementary Tables 8 and 9. Most importantly, Asp6 expresses H-bonds with Lys244 and Glu246 of CTD during > 60 % of the simulation period.

3.4. Binding energy

The C-tail is made up of 18 residues (ITQKDEVTKKDEASPLFA in ASV and DYGKQMAGDDCVASRQDED in HIV) among which about 7 residues have electrically charged side chains. On the other hand, the dimer interface between NTDs and CTDs is reported to be stabilized by hydrophobic interaction (Lodi et al. 1995; Bojja et al. 2011). The binding energy of ASV and HIV IN was calculated in the presence of C-tail and the values are given in Table 2. The HIV IN reaching dimer with G_{bind} of -23.3 ± 11 kCal/mol shows less favorable binding energy than ASV IN models (-42.5 ± 7 and -66.7 ± 9 kCal/mol for ASV and HIV_A, respectively). The more favorable binding energy for the ASV reaching dimer than HIV explains the readiness of wildtype ASV to form a reaching dimer in solution, whereas, substitutions are required to favor a particular dimeric form in HIV. The calculated MM/GBSA binding energy and its components show that electrostatic energy plays a major role in dimerization of ASV and HIV_A IN reaching dimers, while the dimer interface is hydrophobic in nature. In comparison to this, the electrostatic contribution is greatly reduced in HIV IN reaching dimer and is < 50 % of that observed in ASV dimers. Also, the HIV IN reaching dimer shows a more favorable van der Waals energy than the ASV reaching dimers, reinforcing the hydrophobic nature of its dimer interface. Among the HIV reaching dimers, the vdW energy of HIV is comparatively more favorable than HIV_A dimer. Also, the contribution from solvent accessible surface area, which is buried during dimerization, is highly favorable in HIV reaching dimer than ASV dimers implying a stable dimer interface. In the absence of C-tail, the most flexible region of IN, the MM/GBSA binding energy is highly favorable for ASV based models (-65 ± 10 and -88.5 ± 10 kCal/mol for ASV and HIV_A, respectively). Interestingly, the binding energy of HIV reaching dimer is similar in the absence (-23.8 ± 14 kCal/mol) or presence of the C-tail (-23.3 ± 11 kCal/mol).

3.5. Core-core dimer

Numerous studies on HIV IN dimers have reported a core dimer with a CCD-CCD dimer interface (Goldgur et al. 1998; Goldgur et al. 1999; Molteni et al. 2001). This is completely different from the NTD-CTD interface of a reaching dimer and its stability has been analyzed previously (Balasubramanian et al. 2012). The binding energy for CCD-CCD dimerization is highly favorable as -102.42 ± 7 kCal/mol (Table 2). This CCD-CCD dimer interface is also observed when two reaching dimers interact via their CCDs to form a tetramer (Bojja et al. 2013). Due to the very favorable binding energy, the core dimer is highly stable and hence can be observed as stable intermediates in solution. The major difference between core dimer and reaching dimer is the structure of CCD-CTD linker and the orientation of CTD. This linker region is predicted to be disordered in nature and hence can adopt any conformation. When it adopts helical nature, core dimer is formed, while the reaching dimer is obtained when its helicity is disturbed. The spatial orientation of

individual domains in core and reaching dimer forms was extracted by performing DynDom (Hayward & Berendsen 1998; Hayward 1999) analysis (Figure 5 and Table 3). In the initial structures, both NTD and CTD show displacement with respect to the fixed CCD facilitated by the flexible inter-domain linkers (residues 44-58 and 187-207). The NTD shows only translation motion between the two conformations while the CTD experiences a bending motion with respect to the CCD-CTD linker. Major conformational changes between the two structures are expressed by CTD alone. After equilibration, when the structures have relaxed to their environment, the NTD of HIV reaching dimer has moved towards the CCD-CTD linker and reaches a conformation as seen in the core dimer. Hence after relaxation, the displacement of CTD alone is noticeable while the NTD and CCD together form a single fixed domain. For these different spatial orientations of CTD, the hinge residues 209-213 were identified as responsible. The kink at Thr210 identified by previous studies (Chen et al. 2000; Balasubramanian et al. 2012) is also observed to be involved in the transition of a core dimer to a reaching dimer. In contrast to this, both NTD and CTD of HIV_A reaching dimer have to undergo sterically unfavorable rotation, translation and closure motions to attain the core dimer conformation. The flexibility of CCD-CTD linker due to its disordered nature is reported to be important for the proper functioning of IN by positioning the CTD in *cis* and *trans* to the neighboring monomer (Ballandras-Colas et al. 2016; Yin et al. 2016). The area and volume of the dimer interface is also higher in core dimer when compared to the reaching dimers (Table 1). The core dimer is the most stable with a relatively structured CCD-CTD linker, which may adopt a helical/coiled conformation at various stages of HIV life cycle.

3.6. Brownian Dynamics Simulations

MD analyses have clearly depicted the structural preference of the SAXS derived HIV reaching dimer compared to the earlier HIV_A reaching dimer. Also, based on the G_{bind} , it was identified that core dimer interface is energetically more preferable than reaching dimer interface in both dimeric forms. Hence, the present study focuses on identifying the feasibility of HIV IN dimerization via both core and reaching dimer interfaces using Brownian dynamics simulations. The application of Brownian dynamics in calculating the association or dissociation rate constant in protein complex is well reported (Gabdouline & Wade 1998; ElSawy et al. 2010; Mereghetti, Kokh, McCammon & Wade 2011; Pang, Qin & Zhou 2011; ElSawy et al. 2013). In Brownian dynamics, the formation of an encounter complex is generally assessed by any one of the parameters such as buried surface area threshold, RMSD threshold, energy criteria or residue contact criteria (Janin 1997; Gabdouline & Wade 1998; Vijayakumar et al. 1998). Among these parameters, the residue contact criterion provides the rates in good agreement with the experimentally reported rates, when the hydrogen bonding pairs at the interface are considered (Gabdouline & Wade 1997). Accordingly, hydrogen bonding pairs identified at the dimer interface of both core and reaching dimer were considered as the reaction criteria (Supplementary Table 10). The rate of formation of encounter complexes was calculated for both two and three independent contacts. Figure 6 shows the variation in the calculated association rate as a function of donor-acceptor separation. The formation of both core and reaching dimer can be considered as fast diffusional encounters since their rate of association is greater than $10^6 \text{ M}^{-1}\text{s}^{-1}$. The rate of diffusional encounter is enhanced in case of core dimer interface compared to the

reaching dimer interface, and the rate of formation of two contact encounter complex is faster than the three contact complex. Since the total charge of both the IN structures are same, the difference in the rates occur only due to the electrostatic potentials of the dimer interfaces. A two contact diffusional encounter occurs at a closer range, when the proton donor and acceptor atoms are separated beyond 4.0 Å, while three contacts are formed only at a separation of 8.0 Å. Hence, it is clearly observed that formation of HIV IN core dimer is more favorable than the reaching dimer and their encounter complex conformations are shown in Figure 7.

4. Conclusions

The CCD-CTD linker plays a major role in conferring conformational flexibility and stability to IN which is highly important for its various functions. IN can attain various conformations during the viral life cycle to perform a variety of functions. The dynamic nature of IN has posed major problems in identifying these functional forms. The present studies have provided insight into the various conformations that IN may attain during its life cycle. The dimeric structures identified so far are: a core dimer with CCD-CCD dimer interface and a reaching dimer involving NTDs and CTDs at the interface. Though a tetrameric form of IN is identified in PFV, the NTD and CTD of the outer subunits are not mapped. Hence, it can be assumed that IN is highly dynamic and may possess many intermediate conformations. In this study, molecular dynamics simulations and free energy analyses were performed to understand the stability of reaching dimer models of ASV and HIV. The structural dynamics of these models was compared with that of a core dimer. From this study, it was identified that the reaching dimer models of ASV and HIV_A IN are structurally less stable when compared to the HIV reaching dimer identified in 2013 (Bojja et al. 2013). Though HIV_A reaching dimer is energetically favorable, the HIV reaching dimer is structurally favorable. This is due to the fact that in 2013 model, few regions of the CCD-CTD linker attain a helical nature (as predicted from its sequence propensity) and makes the structure highly compact, which further stabilizes the CCDs and prevents their collapse into the dimer interface. Also, the spatial arrangement of individual domains in a monomer of HIV reaching dimer is very similar to the arrangement in a core model only with a difference in the CCD-CTD linker and CTD. The C-tail expresses maximum number of inter-domain H-bonds in HIV reaching dimer. Also, based on the calculated MM/GBSA binding energies, the core dimer is highly stable when compared to all other dimer conformations with a binding energy of -102.42 ± 7 kCal/mol. The buried surface area and volume of the dimer interface are also higher for the interface which involves the CCDs. The rate of association of core interface is higher when compared to the HIV reaching dimer. From these analyses, it is clear that a core-core dimer is more stable than a reaching dimer, and perhaps the different forms of dimers at different energy states might be required for alternate IN functions at different points in the viral life cycle.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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List of abbreviations

DNA	deoxyribo nucleic acid
IN	Integrase
HIV	Human Immunodeficiency Virus
NTD	N-terminal domain
CCD	Catalytic Core domain
CTD	C-terminal domain
ASV	Avian Sarcoma Virus
PFV	Prototype Foamy virus
MMTV	Mouse Mammary Tumor virus
RSV	Rous Sarcoma virus
SAXS	Small angle x-ray scattering

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Figure 1. RMSD (a), RMSF (b), Radius of gyration (c) and Radius of gyration of the dimer interface (d) obtained from the MD simulation of IN reaching dimer structures.

Figure 2. Stability of the π -stacking interaction between Trp259 (in ASV)/Trp243 (in HIV) of both the CTDs obtained from the MD simulation of IN reaching dimer structures.

Figure 3. Structural superimposition of initial (gray) and representative structure (blue) from the most populated cluster of 50 ns MD simulated ASV (a), HIV_A (b) and HIV (c) IN reaching dimers in the presence (i) and absence (ii) of C-tail.

Figure 4.

H-bonds stabilizing the reaching dimer interface extracted from the MD simulations of ASV (a), HIV_A (b) and HIV (c) IN reaching dimers in the presence (i) and absence (ii) of C-tail.

Figure 5.

The spatial arrangement of various domains in a core-core dimer (gray) and reaching dimer. (a) and (b) shows the variation in HIV and HIV_A reaching dimer, respectively. The regions colored in blue are identified as fixed domain relative to which the displacement of regions colored in red (moving domain) are reported. The green colored regions designate the mechanical hinge for the displacement of the moving domains. The arrow represents the plane of motion with the arrow head pointing towards the direction of motion.

Figure 6. Association rate constants for two (a) and three (b) contacts as a function of hydrogen bond donor-acceptor separation for reaching dimer (open circles) and core-core dimer (black circles).

Figure 7.
The encounter complexes of core-core (a) and reaching dimer (b) of HIV.

Table 1

The buried surface area and volume of the dimer interfaces calculated for the initial and representative structure from the most populated cluster of 50 ns MD simulations of all integrase dimers.

Structures	Initial		Representative conformation	
	Area	Volume	Area	Volume
ASV tail	8667	5863	8364	11260
ASV notail	6914	960.8	9483	12130
HIV _A tail	8166	6136	7178	3166
HIV _A notail	7879	829.8	7983	5810
HIV tail	8268	4840	8132	11190
HIV notail	6103	1163	7598	7860
Core dimer	8260	433.8	8831	15720

MM/GBSA binding energy and its components calculated from the last 10 ns simulation of all IN structures.

Table 2

Structures	E_{vdW}^c	E_{ele}^d	E_{polar}^e	$E_{nonpolar}^f$	G_{gas}^g	G_{sol}^h	G_{bind}^i
ASV tail	-129.19 ± 8.7	818.64 ± 91.4	-714.17 ± 88.9	-17.81 ± 1.3	689.44 ± 90.2	-731.97 ± 89.1	-42.53 ± 7.4
ASV notail	-119.21 ± 8.6	745.19 ± 87.5	-674.17 ± 81.5	-16.9 ± 1.1	625.98 ± 86.1	-691.07 ± 81.2	-65.09 ± 10.7
HIV _A tail	-138.47 ± 7.8	-874.13 ± 86.6	966.88 ± 82.2	-21.01 ± 1.2	-1012.59 ± 87.3	945.87 ± 81.4	-66.73 ± 9
HIV _A notail	-113.38 ± 8.8	-794.93 ± 63.3	837.68 ± 60.2	-17.94 ± 0.9	-908.31 ± 63.1	819.74 ± 60	-88.57 ± 9.6
HIV tail	-137.68 ± 8.3	-268.39 ± 48.4	402.19 ± 43.1	-19.42 ± 1	-406.08 ± 46.8	382.77 ± 42.9	-23.31 ± 10.7
HIV notail	-169.59 ± 8.9	-455.68 ± 85.9	627.39 ± 78.5	-25.89 ± 1.	-625.28 ± 87.4	601.49 ± 77.8	-23.79 ± 13.6
Core dimer	-125.9 ± 5.8	-390.88 ± 29.7	433.9 ± 25.3	-19.56 ± 0.6	-516.77 ± 29.6	414.35 ± 25.1	-102.42 ± 7.7

^c van der Waals energy

^d Electrostatic energy

^e Polar component of solvation energy calculated by GB method

^f Non-polar component of solvation energy calculated from SASA

^g gas phase molecular mechanics energy

^h Solvation energy (E_{polar} + $E_{non-polar}$)

ⁱ Binding energy (G_{bind} = G_{dimer} - ($G_{monomerA}$ + $G_{monomerB}$)

Table 3

DynDom extracted relative motions of the HIV and HIV_A reaching dimers with respect to the composite dimer.

Relative domain motions	HIV reaching dimer	HIV _A reaching dimer
Domain 1 - Fixed domain	1-209	55-220
Domain 2 - Moving domain	214-288	1-44, 223-288
Angle of rotation	72.6	121.3
Translation along axis	-12.9	-12.5
% Closure motion	100	10.7
Bending Residues	209-213	45-54, 221, 222