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Binding Modes of Two Novel Dinucleotide Inhibitors of HIV-1 Integrase

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Abstract—Insights into the binding modes on HIV-1 integrase of our novel dinucleotide inhibitors (pisodApdC and pdCpisodU) have been obtained using molecular docking experiments. In contrast to their base-stacked unbound state, these dinucleotides in their integrase-bound state prefer unstacked conformations for a more extensive interaction with the active site. The calculated free energies of binding are in concert with the experimentally acquired anti-HIV-1 integrase data.

One of the enzymes encoded by the *pol* gene of HIV-1 is a 32 kDa protein called HIV-1 integrase (IN).[1,2] This viral enzyme is involved in the integration of HIV DNA into host chromosomal DNA. The biochemical mechanism of integration of HIV DNA into the host cell genome involves a carefully defined sequence of DNA tailoring (3-processing) and coupling (DNA strand transfer or integration) reactions.[1,2] However, unlike major therapeutic advances in the HIV reverse transcriptase and protease areas, there are no drugs for HIV/AIDS in clinical use where the mechanism of action is inhibition of HIV integrase.[3] Thus, new and fundamental knowledge on inhibitors of this enzyme is of critical importance in the anti-HIV drug discovery area. We have synthesized novel dinucleotides,[4] containing one isonucleotide unit,[5] and evaluated their biological properties towards HIV-1 IN. Two of these compounds, pisodApdC and pdCpisodU (Fig. 1), exhibit potent anti-HIV IN activity (Table 1).[4] In order to understand the nature of binding of these inhibitors to the active site of integrase, we have carried out molecular modeling experiments on ligand–enzyme interactions and this communication reports on some of our interesting findings. As there are no X-ray crystal structures available for HIV IN complexed with a dinucleotide, docking experiments could provide useful information on how these inhibitors bind to the active site of the enzyme. The structural and conformational data obtained from the ligand-enzyme complexes could then aid in designing new compounds with improved biological properties.

The active core of HIV-1 IN is defined by three amino acid residues: Asp^{64}, Asp^{116}, which bind to a Mg^{2+} cofactor, and Glu^{152}, which is not coordinated to the metal ion. Values (IC_{50}) obtained previously by us for the inhibition of DNA binding by the dinucleotides are of the same order as the IC_{50} data for inhibition of HIV integrase catalytic activities which suggest that these compounds bind to the catalytic core of integrase.[4] Thus, for the docking experiments it was assumed that the dinucleotides bind within the core domain. This also coincides with the recently reported structure of HIV-1

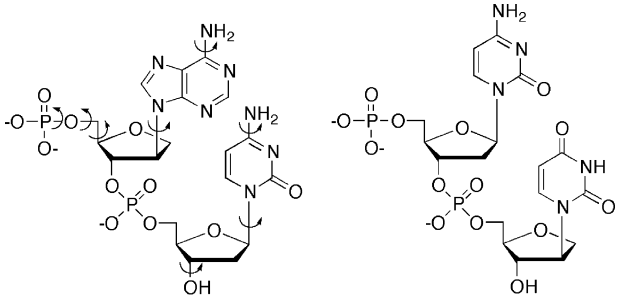

Figure 1. Structures of pisodApdC and pdCpisodU. The curvy arrows show which bonds were rotatable during the docking experiment with stacked bases. Unmarked bonds were rigid. Subsequent runs were carried out with all eligible bonds being rotatable.

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Table 1. Anti-HIV integrase data for pisodApdC and pdCpisodU

Compd	Inhibition of HIV-1 IN (3′-processing) IC_{50}, μM	Inhibition of HIV-1 IN (strand transfer) IC_{50}, μM
pisodApdC	19	25
pdCpisodU	7.5	5.9

IN complexed with 5-CITEP, the first crystal structure of a ligand inside the integrase active site.[6] The software used for our calculations was AutoDock 3.0.[7] It allows automated docking of flexible ligands to an enzyme receptor by rapid energy evaluation with prior calculated grid-based atomic affinity potentials. The Lamarckian genetic algorithm (LGA) was employed to find appropriate binding positions, orientations, and conformations of the ligands. This method is known to be superior to the genetic algorithm without local search and also to the Monte Carlo simulated annealing for docking of small ligands to proteins.[8] The global optimization was started with a population of 100 randomly positioned individuals, a maximum of 2.5×10^6 energy evaluations, and a maximum of 27,000 generations. The crossover rate was set to 80% and the mutation rate to 2% with Cauchy distribution parameters $\alpha=0$ and $\beta=1$. Simple elitism was applied. Pseudo-Solis and Wets local search was performed with a frequency of 6% to each population of a generation and for a maximum number of 3000 steps. The initial local step size ρ was 1. Its value was doubled or halved after a maximum of four consecutive failures or successes. The low bound on ρ for local search termination was 0.01. The number of initial torsions varied depending on ligand and type of experiment. During each experiment 100 runs were carried out and 10 experiments were executed for each ligand. Final data were taken from the experiment that produced the lowest total docking energy.

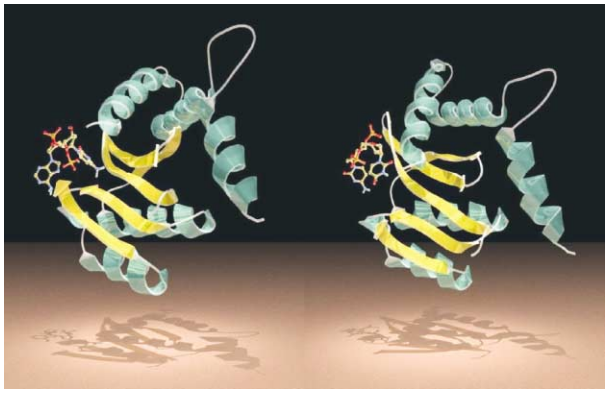

Figure 2. Ribbon diagrams of the B chain of HIV-1 integrase complexed with pisodApdC (left) and pdCpisodU (right). Graphic was prepared with Swiss-PbdViewer v3.7b2 and then rendered with POV-Ray Version 3.1g (WinMegaPov 0.7).

The ligands were created and initially optimized with SYBYL.[9] All protons were added with the exception of the three phosphate protons since the dinucleotides are anionic under physiological conditions. Charges were assigned using the Gasteiger–Hückel method and ligand energies were minimized using the TRIPOS force field. In agreement with the experimental CD and UV data, the ligands seem to prefer a base stacked conformation in the absence of the enzyme. During final preparations Kollman charges were added, nonpolar hydrogens and lone pairs were merged, aromatic carbons identified, and lastly, the rigid root and rotatable bonds were defined.

For the docking, the X-ray crystal structure of HIV-1 IN complexed with Mg^{2+} was used (PDB code: 1biu).[10] All water molecules were removed with PDBDE-WATER, a script distributed with AutoDock. The protein was then further prepared by removing nonpolar hydrogens, merging lone pairs, and addition of Kollman united-atom partial charges. Finally, solvent parameters were added and grid parameters defined. The grid box dimensions were set to $4\times40\times40$ Å centered on the metal ion using a grid spacing of 0.375 Å. The grid

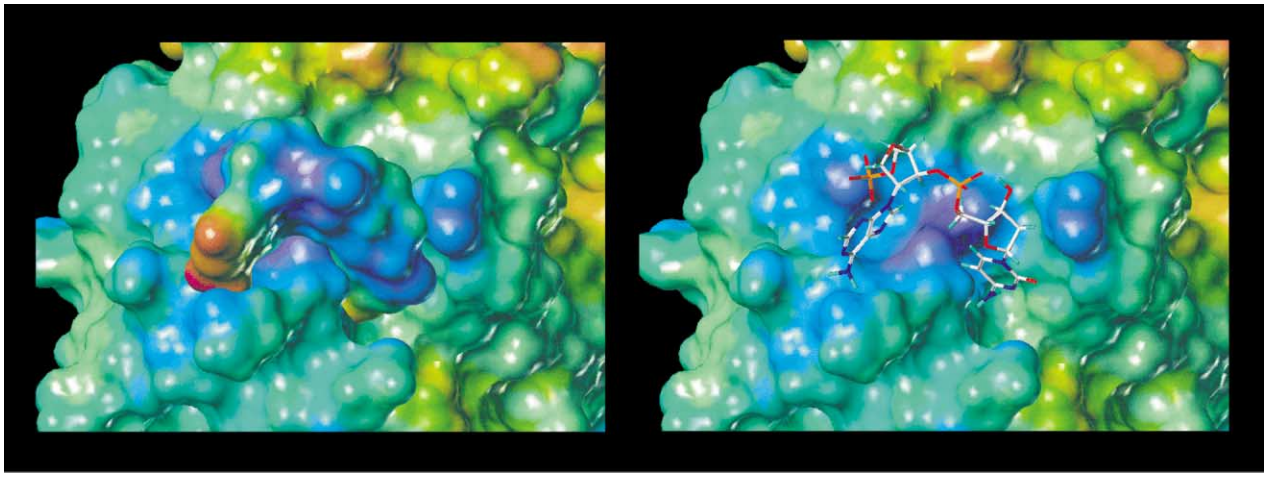

Figure 3. Electrostatic potential surface map of HIV-1 IN complexed with pisodApdC. Shown is the potential binding area of the inhibitor (Connolly surface, left; capped sticks, right). Hydrogens were added to the B chain of the protein and to the ligand (with exemption of anionic functional groups). Conolly surfaces were rendered after addition of charges using the Gasteiger–Hückel method. The color ranges are based on the individual molecule properties to display greater details of potential differences on the surface of enzyme and ligand (high electrostatic potential, red; low electrostatic potential, blue).

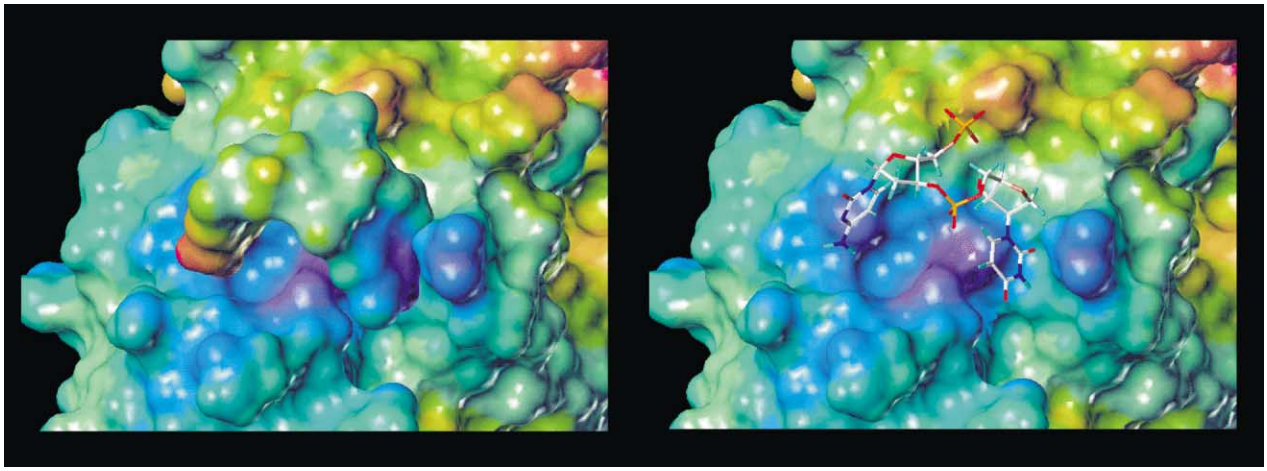

Figure 4. Connolly surface with electrostatic potential maps of pdCpisodU within the HIV-1 IN active site.

maps were subsequently generated with AutoGrid 3.0 (included in the AutoDock 3.0 software package). The first docking experiment was carried out with pisodApdC in stacked base conformation. The bonds between base and sugar moieties, however, were kept in rotatable mode. The results were then compared with the data obtained from runs with all eligible bonds being rotatable. The ligand with more torsional degrees of freedom showed lower final docked energies than the torsionally more restricted one (difference of 3.42 kcal/mol). The stacked form was located closer to the center of the rather wide active site while the flexible dinucleotide was folded into a pocket created by Leu^{63}, Asp^{64}, Asp^{116} and Glu^{152}. This orientation increases the enzyme surface covered by the interacting ligand resulting in tighter binding which is manifested by a lower binding free energy (ΔG_{bind}). A comparable position was found for pdCpisodU (Fig. 2), which was also kept completely flexible. Both dinucleotides seem to prefer a position with one pyrimidine base tucked in the polar channel between Asp^{64} and Glu^{152}. The electrostatic potential surface maps show how each inhibitor fits properly into the active site, which is largely negatively charged (Figs. 3 and 4). This orientation would not be possible with stacked bases and it is very likely that these ligands change their original preferred minimum conformation within the active site to allow greater coverage of enzyme surface and thereby tighter binding.

These new conformations are further stabilized by several hydrogen bonds between the protein and the base or phosphate moieties of the dinucleotides. The 3′-phosphate group of pisodApdC points towards the magnesium cation and can hydrogen bond with His^{114} (Fig. 5). Other hydrogen bonds can be formed with Leu^{63}, His^{67}, and Asn^{117}. The isouridine containing

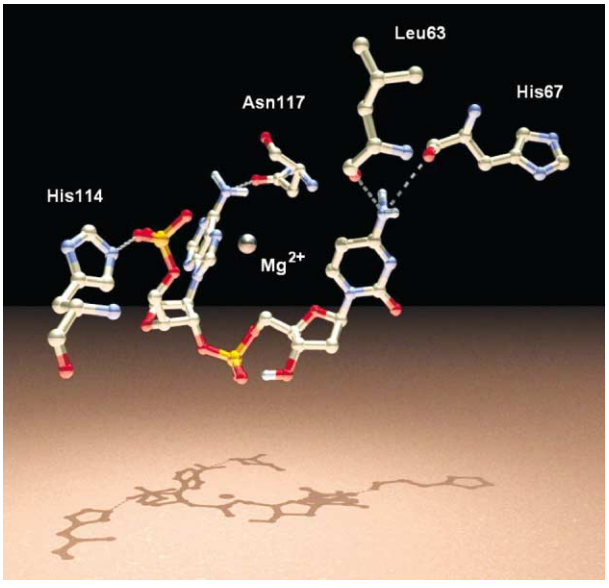

Figure 5. Hydrogen bonding of pisodApdC with HIV-1 IN.

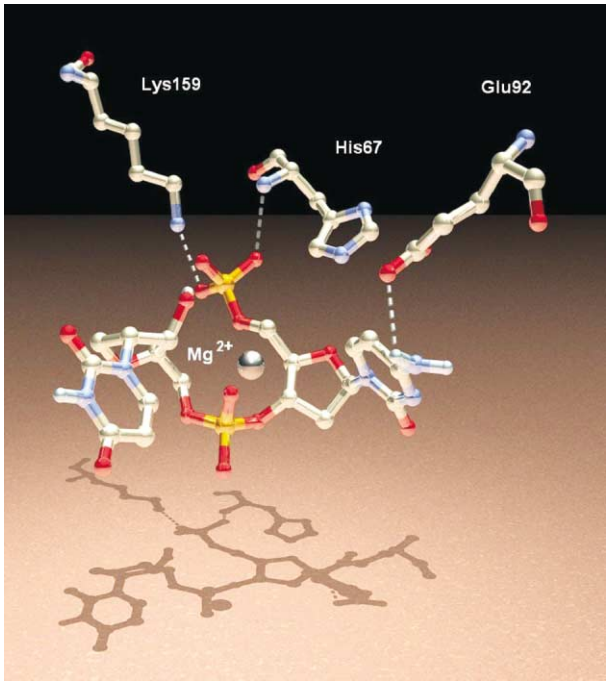

Figure 6. Hydrogen bonds between pdCpisodU and HIV-1 IN.

Table 2. Docking energies of the ligand–enzyme complexes

Ligand	N_{rot}[a]	ΔG_{final}[b] (kcal/mol)	ΔG_{bind}[c] (kcal/mol)
pisodApdC (stacked bases)	8	−9.74	−2.55
pisodApdC (flexible)	13	−13.16[d]	−3.77[d]
pdCpisodU	12	−13.75[d]	−4.43[d]

[a]Number of total rotatable bonds.
[b]Final docked energy.
[c]Estimated free energy of binding.
[d]Values represent lowest result of 10 experiments.

ligand is even closer to the core amino acid triad with the 5′-phosphate group directed towards the metal ion. It can form hydrogen bonds with Glu^{92}, His^{67}, and Lys^{159} (Fig. 6). AutoDock 3.0 predicted a tighter binding mode of pdCpisodU over pisodApdC (Table 2). The lower final docked energy and greater binding free energy of pdCpisodU are in agreement with its lower IC_{50} values.

In summary, molecular modeling docking experiments were performed on our dinucleotide inhibitors using the enzyme structural information obtained from the X-ray crystal structure of HIV-1 IN. New insights into the potential binding modes of these flexible molecules were obtained. The results suggest that, in contrast to their base-stacked unbound state, these dinucleotides in their integrase-bound state prefer conformations in which the bases are not stacked which allows for a more extensive interaction with the enzyme surface including hydrogen bonding with several active site amino acid residues. The free energy data for binding are in remarkable concert with the experimental data on inhibition of HIV-1 integrase.

Acknowledgements

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