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Effects of fluorine substitution of cytosine analogues on the binding affinity to HIV-1 reverse transcriptase

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Abstract—In order to find out the possible role of fluorine substituent in binding of the fluorinated cytidine analogues at the active site of HIV-1 RT, binding modes of several 5-fluoro cytidine analogues, such as FTC, D-dioxolane 5-FC and D-2′F-d4FC to the active site of HIV-1 reverse transcriptase, were compared by molecular dynamics studies.

The addition of a fluorine atom at the 5-position of the pyrimidine ring has been found to alter the kinetic parameters as well as antiviral potency of the corresponding nucleoside analogues.[1] Studies have shown that modifying the 5-position of the cytosine ring with fluorine may reduce toxicity and in some cases increase the antiviral potency.[2–4] In the previous kinetics studies, the 5-fluoro substitution of the triphosphates of (−)-β-L-2′,3′-dideoxy-3′-thiacytidine (3TC) and (+)-β-D-2′,3′-didehydro-2′,3′-dideoxycytidine (d4C) caused a higher overall efficiency of incorporation compared to their unfluorinated counterparts during DNA-directed synthesis (Table 1), suggesting the possible role of 5-fluoro substituent in the binding of the nucleoside triphosphate at the active site of HIV-1 reverse transcriptase (RT).[1] However, it is noteworthy that, while the 5-fluoro substitution of D-d4CTP resulted in remarkable increase in both maximum rates of incorporation (k_{pol}) and equilibrium binding constant (K_d), FTCTPs marginally increased efficiency of incorporation is the result of the increased rate of incorporation (k_{pol}) instead of the increased stability of the enzyme–ligand complex (K_d) (Table 1). Also, in contrast to the rarely negative effect of a 5-fluoro substitution, the 2′-fluoro substitution of D-d4FCTP caused a 13.5-fold decrease in the efficiency of incorporation and two-fold increase in K_d during DNA-directed incorporation (Table 1).

Therefore, even though the fluorine substitution apparently alters the kinetic behaviors of the nucleoside triphosphates, in terms of the binding affinity, it is clear that the effect of fluorine substitution of the nucleoside triphosphate is highly dependent on the substitution position as well as the conformation of the sugar moiety. Thus, it is of interest to investigate the binding modes of the various fluorine-substituted nucleoside triphosphates and correlate it with the effects of the highly electronegative fluorine atom on the equilibrium binding constants (K_d) of the nucleoside triphosphate analogues to the active site of RT. For this purpose, three structurally different fluorinated nucleoside triphosphates such as FTCTP, D-dioxolane 5FCTP and D-2′F-d4FCTP (Fig. 1) were docked into the active site of

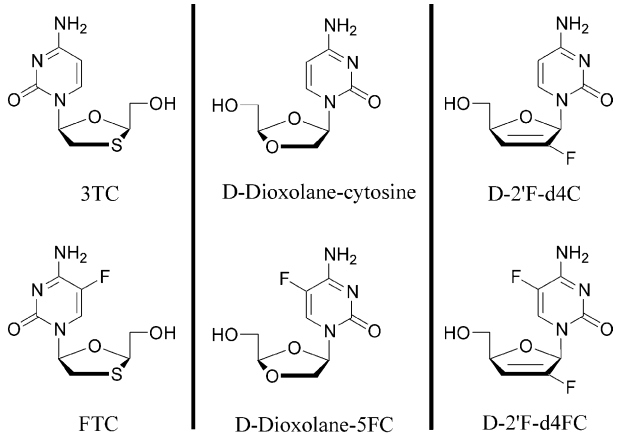

Figure 1. 5-Fluoro cytidine analogues studied.

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HIV-1 RT. After molecular dynamics simulation followed by exhaustive energy minimization, the resulting enzyme–inhibitor complexes were analyzed to provide a dynamic picture of the active site residues of RT which discriminates subtle conformational differences of the incoming nucleoside triphosphate analogues.

Since the change in binding mode of cytidine analogues in response to the isosteric 5-fluorination should influence electrostatic, not steric effects, the analysis was focused on the electronic effect of the fluorine atom.

All molecular modeling of the enzyme-substrate complexes was carried out using Sybyl 6.7 (Tripos, Inc.) and MacroModel 7.0 (Schrödinger, Inc.). As was reported in our previous molecular modeling study of HIV-1 RT,[5] the enzyme site of the enzyme–ligand complex was constructed based on the X-ray structure of the covalently trapped catalytic complex of HIV-1 RT with TTP and primer-template duplex (PDB entry 1rtd).[6] The initial Cartesian coordinates for each inhibitor were generated based on the X-ray coordinates (D-dioxolane cytidine[7] and D-d4C) or conformational analysis (FTC).[8] Each RT·DNA·nucleoside triphosphate complex was minimized until there was no significant movement in atomic coordinates using MMFF94s force field in the presence of GB/SA continuum water model before performing molecular dynamics simulations. A conjugate gradient, Polak-Ribiere 1st derivative method, was used for energy minimization. Molecular dynamics simulations on RT·DNA·nucleoside–TP complex was performed with MMFF94s in the presence of GB/SA continuum water model on a Silicon Graphics Octane2 workstation running the IRIX 6.5 operating system by heating from 0 to 300 K over 5 ps and equilibrating at 300 K for an additional 10 ps. Production dynamics simulations were carried out for 500 ps with a step size of 1.5 fs at 300 K. A shake algorithm was used to constrain covalent bonds to hydrogen atoms. For simulation of the RT·nucleoside–TP complex, the residues further away than 15 Å from the active site were not considered and the residues from 6 to 15 Å were constrained by harmonic constraints. Only residues inside 6 Å sphere from the bound nucleoside–TP were allowed to move freely.

Table 1. Efficiency of cytidine analogue incorporation into a DNA primer directed by a DNA template[1]

Compd	k_{pol} (s^{-1})	K_d (μM)	Efficiency[a] ($s^{-1}\mu M^{-1}$)
3TCTP	0.019	15	0.0013
FTCTP	0.039	12	0.0033
D-d4CTP	0.16	18	0.0086
D-d4FCTP	0.95	31	0.031
D-2′F-d4FCTP	0.15	64	0.0023

[a] k_{pol}/K_d.

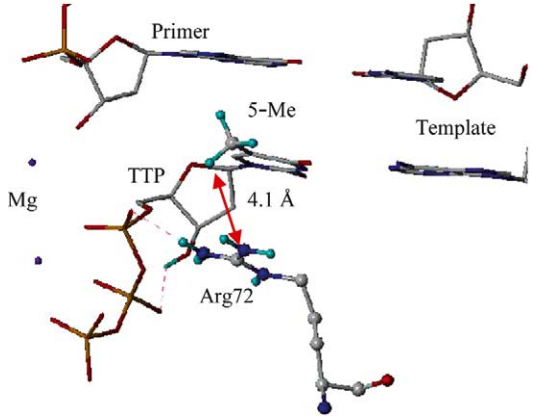

Figure 2. Crystal structure of the active site of RT.

Fluorine can often be used to isosterically replace a hydroxyl group because of its similar polarity to oxygen and the ability to be a hydrogen bond acceptor. Fluorine is also the third smallest element and can serve as a replacement for hydrogen on a methylene unit without a large steric change, possibly endowing novel electrostatic interactions to the modified compound such as hydrogen bonding interactions at the enzyme active site. The changes of the binding affinities of the cytidine analogues in response to 5-fluorination may be attributable to an interaction with Arg72 in the RT active site because the Arg72 is the only amino acid within 5 Å of the anticipated position of the 5-fluorine (similar to the position of the methyl unit of thymidine in the crystal structure of RT bound to TTP and primer/template) (Fig. 2).[6]

The presence of three nitrogens in Arg72, one of which interacts with the triphosphate moiety of TTP by a hydrogen bond along with the fluorine's ability to be a hydrogen bond acceptor, may allow Arg72 to have an

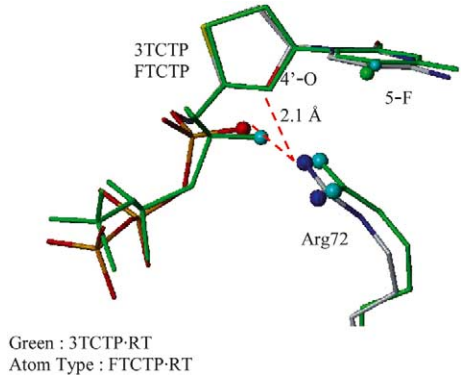

Figure 3. RT FTCTP versus RT· 3TCTP.

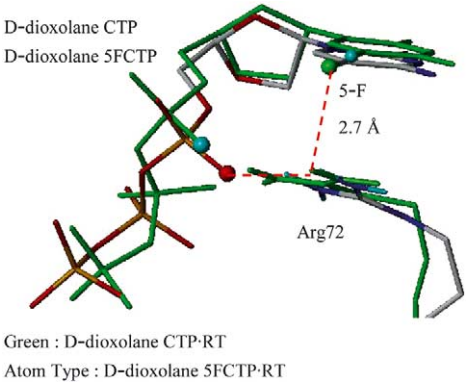

Figure 4. RT·D-dioxolane cytoside versus RT·D-dioxolane 5FC.

additional contact with the base moiety of the nucleoside analogue at the active site of RT. However, after molecular dynamics simulation, RT-FTCTP complex maintained almost same conformation as that of RT-3TCTP because Arg72 was found to be interacting not with 5-fluorine atom but with the α-phosphate as well as 4′-oxygen atom in the oxathiolane sugar moiety (Fig. 3). By virtue of the unnatural L-sugar conformation, the 4′-oxygen atoms in both 3TCTP and FTCTP are located in close proximity to Arg72. Therefore, energetically, RT-FTCTP and RT-3TCTP were indistinguishable, which is consistent with the similar equilibrium binding constants of 3TCTP and FTCTP (Table 1).

In spite of the lack of the kinetic data for the incorporation of D-dioxolane cytosine and the corresponding 5-fluoro analogue, the molecular dynamics study expects that the D-dioxolane 5-FC would be energetically favored over its cytosine analogue mainly due to the electrostatic interaction between 5-fluorine atom and ε-amino group of Arg72, which shows significant conformational difference compared with the binding mode of D-dioxolane cytosine (Fig. 4). Unlike 3TC/FTC, the natural D-conformation of D-dioxolane nucleoside does not allow the interaction between the 3′-oxygen atom in the dioxolane sugar ring with Arg72, which enables Arg72 to readily donate hydrogen bonding to the fluorine atom at 5-position of the cytosine base (Fig. 4).

In the case of D-2′F-d4FC, Arg72 is involved in the electrostatic interaction with the 2′F substituent, instead of the fluorine atom at the 5-position. Therefore, compared with the 3TC/FTC binding, Arg72 is located deep inside of the active site (Fig. 5).

As a result, the conformation of D-2′F-d4C/D-2′F-d4FC triphosphate is quite different from that of 3TC/FTC triphosphate in which the α phosphate is hydrogen bonded to Arg72. As a result of this conformational change, one of the two Mg atoms shifts outside of the YMDD motif, resulting in a distorted binding mode (Fig. 6) and thereby reduced binding affinity to the RT. However, the unfavorable effect of 2′-fluorine substitution at the 2′,3′-unsaturated nucleosides can be partially compensated by the hydrophobic interaction of the 2′,3′-double bond with Tyr115 which forms a bottom of the so called '3′-OH pocket' (Fig. 7). Tyr115 is known to exclude the binding of RNA nucleotides by sterically disallowing the presence of a 2′-hydroxyl and this has lead it to be called the 'steric gate' of RT.[9] However, according to our modeling studies, the Tyr115 is positively contributing for the binding of 2′-F-d4 compound through π-π interaction between the aromatic ring of the Tyr115 residue and the double bond of the fluorovinyl moiety of 2′F-d4N nucleosides (~3.4 Å) (Fig. 7). In our previous study,[10] this kind of interaction was found in almost every d4 nucleosides, and the electronegative 2′-fluoro substitution strengthens this interaction. In addition, the previously noted increased stability imparted to nucleoside analogues by 2′-substitutions[11] may outweigh the negative effects.

In summary, by molecular dynamics studies, we have found that formation of a hydrogen bond between fluorine-substituted nucleoside analogues and Arg72 may provide additional favorable contact within the active site of RT. However, as Arg72 is heavily involved in the stabilization of the triphosphate moiety of the bound nucleoside triphosphate, its interaction with the 5-fluorine atom on the cytosine ring was observed only in a limited case (D-dioxolane-5FCTP).

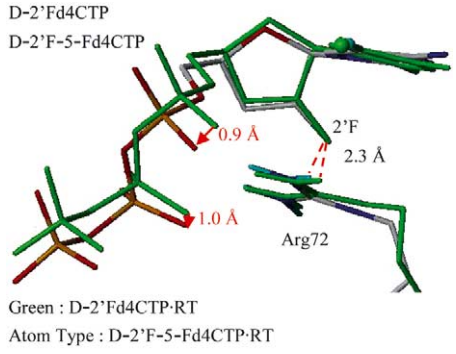

Figure 5. RT·D-2′Fd4CTP versus RT·D-2′F-d4FCTP.

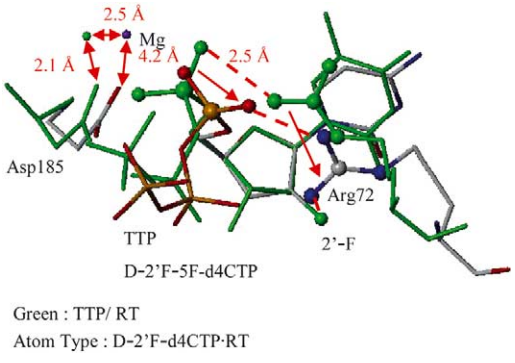

Figure 6. Conformational change at the active site.

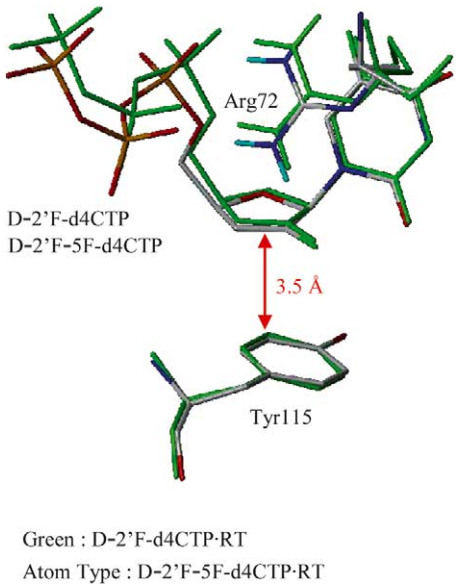

Figure 7. Hydrophobic interaction.

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