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Structures of HIV-1 reverse transcriptase with HBV-associated triple mutations in complex with chirally-distinct nucleoside analogues, ETV-TP and 3TC-TP

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1 Introduction

Hepatitis B virus (HBV) reverse transcriptase (RT) is a crucial enzyme for viral replication, and several nucleoside analogues RT inhibitors are currently used for anti-HBV treatment. Chirally-distinct nucleoside analogues RT inhibitors, entecavir (ETV) and lamivudine (3TC), are major anti-HBV drugs, while common drug resistant amino acid substitutions in HBV RT (M204V/I) are known to emerge in individuals given ETV/3TC. Because the structures of ETV and 3TC are considerably different, it is of interest how ETV/3TC are bound to HBV RT and why the common M204V/I mutations decrease the ETV/3TC susceptibility. We have shown that HIV-1 with HBV-associated triple mutations (Q151M/Y115F/F116Y) in its RT is highly susceptible to both ETV/3TC [1]. In addition, the introduction of M184V (M204V in HBV RT) renders HIV-1$^{Q151M/Y115F/F116Y}$ resistant to ETV/3TC [2]. Thus, we have undertaken the structural studies of HIV-1 RT$^{Q151M/Y115F/F116Y}$ with 3TC/ETV to explore the mechanism of 3TC/ETV binding and of drug resistance conferred by M184V/I.

2 Experiment

HIV-1 RT with mutations Q151M, Y115F and F116Y was overexpressed using *Escherichia coli* and purified by Ni-affinity/ion-exchanging chromatography as previously described [1]. The modified DNA aptamer [3] was used for accommodation of dNTP/NRTI at the active site of RT. After RT:DNA complex formation, the sample was further purified by gel-filtration. HIV-1 RT$^{Q151M/Y115F/F116Y}$:DNA binary complex was crystallized using reservoir solution containing bis-Tris-HCl pH 6.0, ammonium di-hydrogen citrate, $MgCl_2$, PEG 6000, glycerol and sucrose. The crystals were soaked into the cryoprotectant solution supplemented with ETV-triphosphate (ETV-TP)/3TC-triphosphate (3TC-TP). The X-ray diffraction data were collected at BL-1A/17A, PF. The crystals belong to the rhombohedral space group $H3$, with unit-cell dimensions $a = b = 284$ and $c = 98$ Å. The data were processed with the program XDS. The model refinement was performed using the programs REFMAC5 and Phenix.

3 Results and Discussion

The structures of HIV-1 RT$^{Q151M/Y115F/F116Y}$ complexed with DNA:ETV-TP and DNA:3TC-TP were determined at 2.32 Å and 2.51 Å resolution, respectively [2]. The structures revealed that the L-nucleoside analogue 3TC-TP binds in an atypical tight binding conformation dissimilar to the common D-form nucleosides ETV-TP and other dNTPs reported to date. Moreover, the structures have shown that both cyclopentyl methylen of ETV-TP and oxathiolane sulfur of 3TC-TP directly push the Met184 side chain. These results provide structural basis for common mechanism of 3TC/ETV resitance by steric clash between methylene/oxathiolane of ETV/3TC and M184V/I side-chain (Fig. 1).

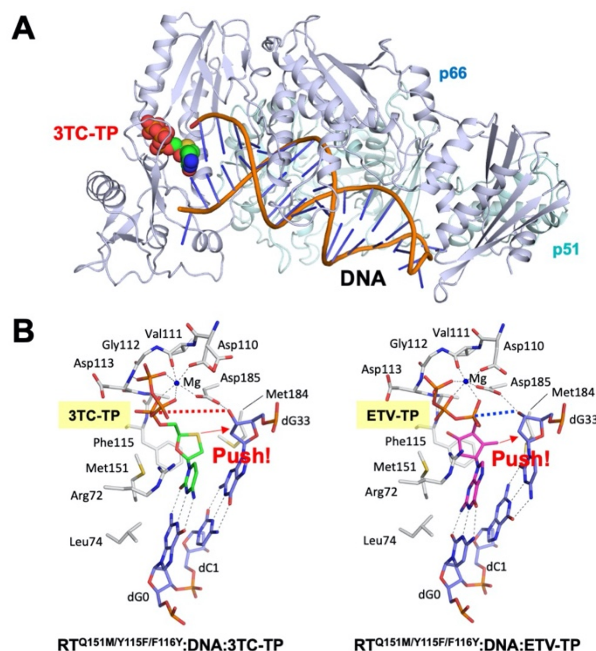

Fig. 1: HIV-1 RT$^{Q151M/Y115F/F116Y}$:DNA:3TC-TP/ETV-TP tertiary complex. (A) Overall structure. (B) Active site structure with bound 3TC-TP/ETV-TP. Met184 (Met204 in HBV) side chain is pushed backward by oxathiolane sulfur and exocyclic methylene of 3TC-TP and ETV-TP.

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