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Structures of HIV-1 reverse transcriptase with HBV-associated septuple mutations in complex with DNA and ETV-TP

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1 Introduction

Hepatitis B virus (HBV) reverse transcriptase (RT) is an essential enzyme for viral life cycle, and all of the approved direct-acting chemotherapeutics for anti-HBV treatment to date are HBV RT inhibitors. Nevertheless, the three-dimensional structure of HBV RT has not been determined due to its notorious insoluble property, and thus the mechanisms of anti-HBV drug binding to HBV RT and of drug resistance by mutations in HBV RT are less clear. We recently showed that HBV-associated Q151M mutation in human immunodeficiency virus type I (HIV-1) RT renders HIV-1 highly sensitive to entecavir (ETV), a potent anti-HBV agent. This finding indicated that the HIV-1 RT with HBV-mimicking mutations at the active site might be useful to explore putative structure of HBV RT active site in the complex state with HBV RT inhibitors. In this study, we have newly designed HIV-1 RT mutant carrying up to seven amino-acid substitutions so as to contain complete reproduction of HBV RT sequences at the active site region. Unfortunately, such septuple amino-acid substitutions impair viral replication and thus the anti-viral assay could not be performed, whereas the recombinant HIV-1 RT mutants could be stably produced with retaining RT activity.

2 Experiment

HIV-1 RT carrying mutations Q151M, G112S, D113A, Y115F, F116Y, F160L and I159L (RT^{7MC}) was overexpressed using *Escherichia coli* BL21(DE3)-RIL, and purified by Ni-affinity and ion-exchanging chromatography as previously described [1]. The hairpin template-primer DNA aptamer [2] was used for accommodation of dNTP/NRTI at the active site. After RT:DNA complex formation, the sample was further purified by gel-filtration chromatography [1]. HIV-1 RT^{7MC}:DNA binary complex was crystallized by hanging-drop vapor-diffusion technique at 20°C using reservoir solution containing bis-Tris-HCl pH 6.0, ammonium di-hydrogen citrate, $MgCl_2$, PEG 6000, glycerol and sucrose. Prior to X-ray diffraction experiments, the crystals were soaked into the cryoprotectant solution supplemented with ETV-triphosphate (ETV-TP)/dGTP. The X-ray diffraction data were collected at the beamline BL-1A, PF. The crystals belong to the rhombohedral space group *H*3, with unit-cell dimensions $a = b = 284$ and $c = 98$ Å. The data were processed with the programs XDS and AIMLESS. The model refinement was performed using the programs REFMAC5 and Phenix.

3 Results and Discussion

The structures of HIV-1 RT^{7MC} in complex with DNA:dGTP and DNA:ETV-TP were determined at 2.43 Å and 2.60 Å resolution, respectively [3]. Two RT molecules in the asymmetric unit are well superimposed with each other with main chain rmsd of below 0.7 Å. Simulated annealing omit map for the bound substrate dGTP and ETV-TP was obvious (Fig. 1), and as shown in the previous structural study of HIV-1 RT^{Q151M}, ETV-TP is bound to the active site of RT^{7MC} with its exocyclic methylen pushing directly the side-chain of Met184 backward. The Met184 of HIV-1 RT corresponds to Met204 in HBV RT, and the M204I/V is known as a major ETV resistant mutation in HBV RT. The present structure of HIV-1 RT^{7MC} strongly suggests that the M204I/V mutation might disrupt the fitness for the methylen group of ETV-TP at the active site of HBV RT.

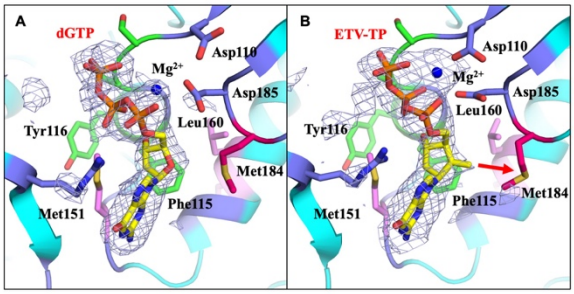

Fig. 1: Simulated annealing omit map for the bound dGTP (A) and ETV-TP (B) at the active site of HIV-1 RT^{7MC}. The maps were contoured at 2.2σ level. The side-chain of Met184 moved backward by exocyclic methylene of ETV-TP as shown by red arrow.

In addition, structural comparison with previously determined wild-type and Q151M mutant of HIV-1 RT showed that slight but significant conformational rearrangements of the amino-acid side chain occurred at the active site of RT^{7MC} (Fig. 2). This structural feature of the RT^{7MC} active site plausibly explain the observed difference between HIV-1 and HBV susceptibility to 4′-modified nucleoside analogues. EFdA, a strong anti-HIV 4′-ethynyl nucleoside analogue, is not potent against HBV. The theoretical modeling of 4′-ethynyl at the active site of RT^{7MC} suggests that the sever steric clash could occur between Leu160 side chain and 4′-ethynyl group. In contrast, 4′-cyano nucleoside analogues such as CAdA are known to be potent against both HBV and HIV-1. The modeling of the cyano group indicates that the expected position of cyano nitrogen would be compatible with both Leu/Phe160 without sever steric clashes. These observations suggest that the structure of the RT^{7MC} active site could experimentally simulate the structure of HBV RT active site.

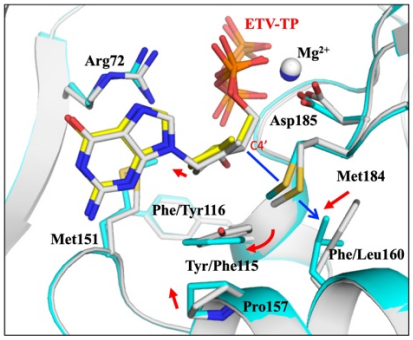

Fig. 2: Conformational rearrangements observed in the active site structure of RT^{7MC}. The movements of the residues 160, 115, and 157 side-chains and the bound ETVTP are represented by red arrows. The expected direction of the C4′-ethynyl/cyano group is also indicated by blue arrow.

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