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HIV-1 reverse transcriptase complexed with DNA and ETV-TP, an anti-HBV drug

Yoshiaki Yasutake[1,*], Shin-ichiro Hattori[2], Hironori Hayashi[2], Kouki Matsuda[2], Noriko Tamura[1], Satoru Kohgo[2,3], Kenji Maeda[2], Hiroaki Mitsuya[2,4,5]

[1]Bioproduction Research Institute, National Institute of Advanced Industrial Science and Technology (AIST), Sapporo 062-8517, Japan

[2]National Center for Global Health and Medicine (NCGM) Research Institute, Tokyo 162-8655, Japan

[3]Faculty of Pharmaceutical Sciences, Sojo University, Kumamoto 860-0082, Japan.

[4]National Cancer Institute, National Institutes of Health (NIH), Bethesda, MD 20892, USA.

[5]Kumamoto University Hospital, Kumamoto 860-8556, Japan.

1 Introduction

Hepatitis B virus (HBV) affecting approximately 400 million people worldwide causes acute and chronic hepatitis, resulting in approximately one million deaths per year [1]. HBV reverse transcriptase (RT) is a vital protein for viral replication and is thus an important target protein for anti-HBV drug development. Nonetheless, obtaining soluble and catalytically active recombinant HBV RT for structural studies remains extremely challenging [2]. In contrast, human immunodeficiency virus type-1 (HIV-1) RT has been known as a stable protein, and the structural analyses of HIV-1 RT have been extensively investigated to date. Although amino-acid sequence similarity between HBV and HIV-1 RTs are very low (~8%), the residues creating the dNTP/drug-binding site (N-site) are moderately conserved. It is thus considered that there are structural and mechanistic analogies between HBV and HIV-1 RT N-site. We have created various HIV-1 RT mutants that mimics HBV-RT N-site, and we found that Q151M mutation in RT becomes HIV-1 highly susceptible to entecavir (ETV), one of the potent anti-HBV RT nucleoside analogue inhibitors. In this study, we have determined the X-ray structure of HIV-1 RT Q151M:DNA in complex with ETV-triphosphate (ETV-TP) or dGTP. The structures provide insights into the mechanism of ETV-TP binding to the RT N-site, and clues to develop new drugs to overcome the reported ETV-resistance of HBV RT.

2 Experiment

HIV-1 RT Q151M was overexpressed by *Escherichia coli*, and purified by Ni-affinity and ion-exchanging chromatography as described [3]. After RT:DNA complex formation, the sample was further purified by gel-filtration chromatography [4]. HIV-1 RT Q151M:DNA binary complex was crystallized by hanging-drop vapor-diffusion method. Prior to X-ray diffraction experiments, the crystals were soaked into the cryoprotectant solution supplemented with ETV-TP/dGTP. The X-ray diffraction data sets were collected at the beamline BL-1A or BL-17A of PF. The crystals belong to the rhombohedral space group *R*3, with unit-cell parameters $a = b = 284$ and $c = 98$ Å. The data were indexed and scaled with the program XDS. The structures were determined by molecular replacement with the program MOLREP, using the previously reported RT:DNA binary complex as a search model (PDB code, 5d3g) [5]. The atomic coordinates and temperature factors were refined with the program Phenix.

3 Results and Discussion

The structures of HIV-1 RT Q151M:DNA in ligand-free, in complex with dGTP or ETV-TP were determined at a resolution of 2.60, 2.38 and 2.45 Å, respectively (Fig. 1).

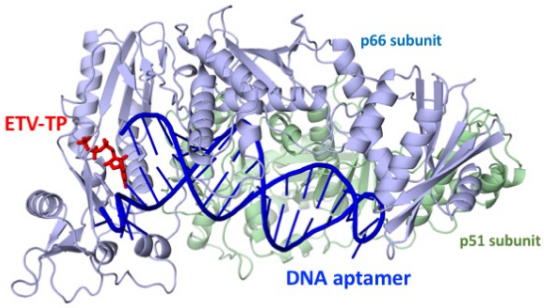

Fig. 1: Overall structure of HIV-1 RT Q151M:DNA:ETV-TP ternary complex

There are two p66-p51 RT heterodimers in the asymmetric unit of the rhombohedral *R*3 lattice. The final refined model contains amino acid residues 3–553 for chains A/C (p66 subunit), 4–427 for chains B/D (p51 subunit) except 214–230 owing to poor electron density, and two DNA chains (nucleotides from –1 to 33 for chain E and from –4 to 33 for chain F).

The structures revealed that the postion and orientation of the bound ETV-TP/dGTP are similar to those in the previously reported HIV-1 RT:DNA:dNTP complex, while there are slight but significant structural differences at the N-site occupied by ETV-TP. First, the ribose-analogous cyclopentane ring of the bound ETV-TP lies slightly apart from the 3′-end nucleotide of the primer DNA. Second, relocation of the side-chain of Met184 occurs by pressing with the protruded exocyclic methylene group of the ETV-TP (Fig. 2). The structures explain the putative ETV-TP binding mechanism to RT, and also the

reasoning for why M204V of HBV RT (M184V in HIV-1 RT) enables ETV resistant: the loss of hydrophobic interactions between the methylene group of ETV-TP and the Met184 side-chain could significantly decrease the binding affinity of ETV-TP at the RT N-site. Based on these results, we propose that modification of the ETV methylene moiety may represent a strategic candidate to develop new anti-viral agents for overcoming ETV resistance.

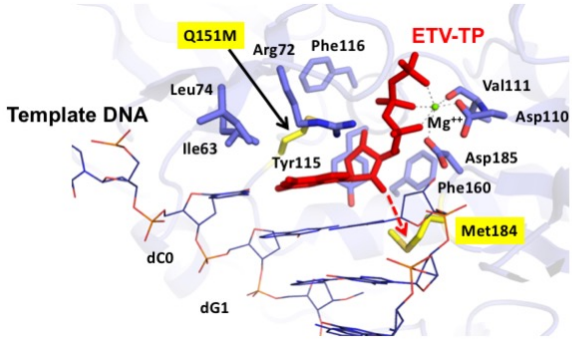

Fig. 2: N-site structure of HIV-1 RT Q151M with bound ETV-TP

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*y-yasutake@aist.go.jp