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Crystal structure of the HIV-1 reverse transcriptase Q151M mutant

Akiyoshi Nakamura[1], Noriko Tamura[1], and Yoshiaki Yasutake[1*]
[1]Bioproduction Research Institute, National Institute of Advanced Industrial Science and Technology (AIST), Sapporo 062-8517, Japan

1 Introduction

Hepatitis B virus polymerase (HBV Pol) is an important target for anti-HBV drug development; however, its low solubility and stability *in vitro* has hindered detailed structural studies. Certain nucleotide reverse transcriptase (RT) inhibitors (NRTIs) such as tenofovir and lamivudine can inhibit both HBV Pol and HIV-1 RT, leading to speculations of structural and mechanistic analogies between the dNTP-binding sites of these enzymes [1]. The Q151M mutation in HIV-1 RT, located at the dNTP-binding site, confers resistance to various NRTIs, while maintaining sensitivity to tenofovir and lamivudine [2]. The residue corresponding to Gln151 is strictly conserved as methionine in HBV Pol. Therefore, the structure of the dNTP-binding pocket of the HIV-1 RT Q151M mutant may reflect that of HBV Pol. To investigate the mutational effect on HIV-1 RT and the structural details of the HBV Pol dNTP-binding site, we performed a crystallographic study of HIV-1 RT Q151M [3].

2 Experiment

HIV-1 RT is composed of p66 and p51 subunits. HIV-1 RT p66_Q151M mutant and p51 were co-expressed in *Escherichia coli* and purified by Ni-affinity and anion-exchanging chromatography. The well-diffracting crystals were obtained with the reservoir solution containing 0.2 M imidazole (pH 8.0) and 9% (w/v) PEG 8000 at 20°C. X-ray diffraction data set of HIV-1 RT Q151M was collected at the beamline BL-17A of the Photon Factory. The raw image data were processed using *HKL*-2000 package. The structure of HIV-1 RT Q151M was solved by molecular replacement using *Molrep* by using an inhibitor-bound HIV-1 RT structure (PDB code, 1RTH) as a search model. The atomic model was rebuilt and modified manually, using the program *Coot*. Model refinement was performed using the programs *REFMAC5* and *phenix.refine*. Finally, R_{work}- and R_{free}-factors were converged to 20.3% and 23.9%, respectively.

3 Results and Discussion

The crystal structure of HIV-1 RT Q151M was determined at 2.6 Å resolution, in a new crystal form with the space group *P*321. Asymmetric unit contains one p66/p51 heterodimer. Although the structure of HIV-1 RT Q151M is well superimposed onto that of HIV-1 RT in a closed conformation, a slight movement of the β-strands (β2-β3) that partially create the dNTP-binding pocket was observed. This movement might be caused by the introduction of the bulky thioether group of Met151.

The dNTP-binding site of HIV-1 RT Q151M was compared with azidothymidine triphosphate (AZT)-bound and tenofovir diphosphate (TNV)-bound HIV-1 RT structures (Fig. 1). The structural comparison suggests that Q151M mutation might cause local structural changes at the dNTP-binding site and the hydrogen-bonding network between amino acids and inhibitors cannot be formed, thereby leading to alterations in the binding affinities for NRTIs. In addition, the hydrophobic interactions between the methyl group of TNV and the thioether group of methionine might compensate for the loss of hydrogen bonds; hence, tenofovir exhibits an inhibitory action on both HIV-1 RT Q151M and HBV Pol.

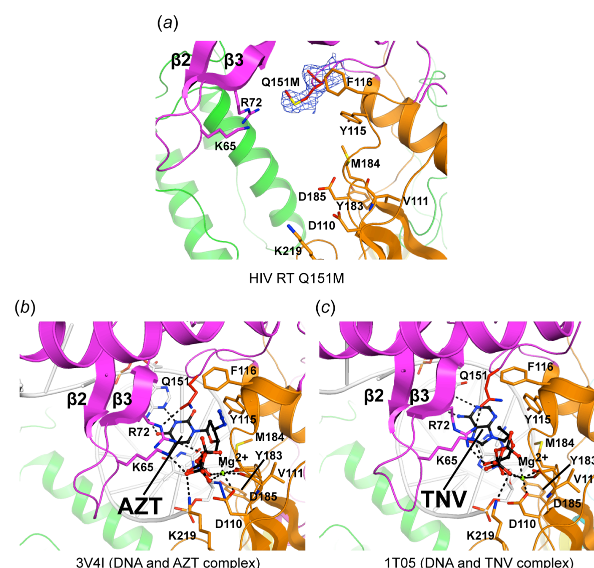

Fig. 1: Comparison of the dNTP-binding site. (a), (b), and (c) show the dNTP-binding pocket of HIV-1 RT Q151M, HIV-1 RT with bound DNA and AZT, HIV-1 RT with bound DNA and TNV, respectively. Residues that form the dNTP-binding site are displayed as stick models. The final 2*mFo*-*DFc* map of the Met151 is shown in blue. Dash lines indicate hydrogen bonds in the dNTP-binding pocket.

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References

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* y-yasutake@aist.go.jp