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Crystallographic analysis of HIV-1 reverse transcriptase with HBV-associated and drug resistant mutations Q151M/Y115F/F116Y/M184V/F160M

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1 Introduction

While hepatitis B virus (HBV) polymerase (Pol) is a vital enzyme for viral replication, its three-dimensional structure has not been determined to date owing to its notorious insolubility. HBV Pol contains four distinct domains: terminal protein (TP), spacer (SP), reverse transcriptase (RT), and RNase H (RH) domains. Among its four domains, HBV RT domain displays less than 25% homology to human immunodeficiency virus type-1 (HIV-1) RT. However, there are various spots, where functionally important (e.g., RT's active site) amino acid residues are well conserved between HBV RT and HIV-1 RT. We have previously shown that HIV-1 with the three HBV-RT-associated amino-acid substitutions (3MB: Q151M/Y115F/F116Y) is hypersensitive to the anti-HBV nucleoside analogs entecavir (ETV) and lamivudine (3TC). Introduction of additional mutations M184V and F160M, which are known to confer ETV/3TC-resistance on HBV RT (M204V and L180M in HBV RT), significantly decreases the susceptibility of HIV-1 RT^{3MB} to ETV and 3TC. To understand the mechanism of ETV/3TC resistance conferred by M184V/F160M mutations, we determined the structures of HIV-1 $RT^{3MB/M184V/F160M}$ in the presence of 3TC-triphosphate (3TC-TP) and dCTP.

2 Experiment

HIV-1 RT with mutations Q151M, Y115F, F116Y, M184V, and F160L ($RT^{3MB/M184V/F160M}$) was expressed in *Escherichia coli* BL21(DE3)-RIL, and purified by Ni-affinity and ion-exchange chromatography as described previously [1]. A template-primer mimetic DNA aptamer [2] was used for structural analysis of the RT:DNA complex. After mixing $RT^{3MB/M184V/F160M}$ and DNA, samples were further subjected to gel-filtration chromatography [1]. The HIV-1 $RT^{3MB/M184V/F160M}$:DNA complex was crystallized by the hanging-drop vapor-diffusion technique at 20°C, using a reservoir solution containing bis-Tris-HCl pH 6.0, 20-40 mM ammonium di-hydrogen citrate, 20 mM $MgCl_2$, 2–4% PEG 6000, 4.8% glycerol, and 2.4% sucrose. Crystals were soaked in a cryoprotectant solution consisting of 25% glycerol, 15% PEG 6000, and 15% sucrose supplemented with 3TC-TP/dCTP, then flash-cooled using liquid nitrogen at 100 K.

3 Results and Discussion

X-ray diffraction data and refinement statistics for HIV-1 $RT^{3MB/M184V/F160M}$:DNA:3TC-TP and $RT^{3MB/M184V/F160M}$:DNA:dCTP are summarized in Table 1.

Table 1: X-ray diffraction data and model refinement statistics

	$RT^{3MB/M184V/F160M}$: DNA:3TC-TP	$RT^{3MB/M184V/F160M}$: DNA:dCTP
BL	BL-1A	BL-1A
Space group	H3	H3
Unit cell (Å, deg)	a = b = 284.2, c = 95.9	a = b = 285.7, c = 96.3
Resolution (Å)	2.57 (2.62-2.57)	2.67 (2.83-2.67)
R_{meas}	0.078 (0.892)	0.089 (0.982)
I/σ	14.5 (2.0)	14.8 (2.0)
Completeness (%)	99.8 (96.3)	100.0 (99.6)
Multiplicity	5.4 (5.1)	5.4 (5.5)
R_{work}/R_{free}	0.192/0.230	0.178/0.226

The structures of HIV-1 $RT^{3MB/M184V/F160M}$:DNA:(dCTP or 3TC-TP) were determined at resolutions of 2.67 and 2.57 Å, respectively. The overall structure of the mutant RT is very similar to a previously reported ternary structure of HIV-1 RT complexed with DNA and dNTP in closed conformation.

The observed electron density for bound dCTP/3TC-TP was very clear (Fig. 1). The bound dCTP in the present structure deviated slightly from the tightly bound position commonly observed in previously reported structures of HIV-1 RT in complex with dNTP. It is likely that the deviation occurs due to the emergence of a Phe115 bulge caused by the introduction of a bulky Met at position 160. The observed structural change is

consistent with enzyme kinetic data for HBV RT, wherein Km values for the dNTP increase with the introduction of L180M/M204V mutations [3]. In contrast, the bound 3TC-TP deviates more from the dCTP, and electron densities for triphosphate and Mg^{2+} are completely missing. It is likely that the highly altered mode of 3TC-TP binding is caused by steric clash between the Phe115/Met160/Val184 bulge, and the bulky sulfur atom in the oxathiolane of 3TC. This is the first experimental report (not *in silico* modeling) of an RT structure with the drug resistant mutations M184V/F160M, thus our data may serve as a model for the design of new agents to overcome drug resistance.

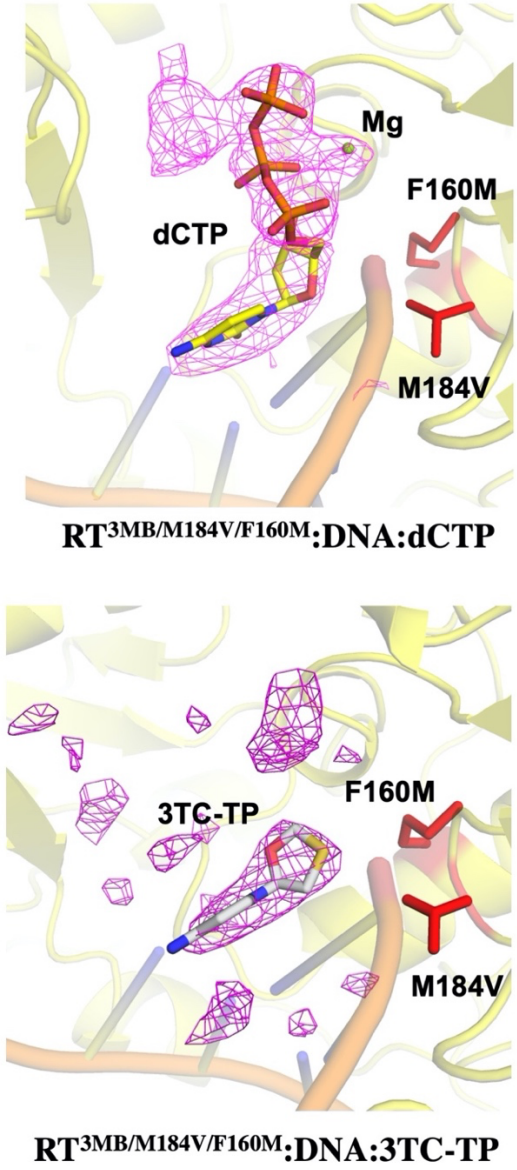

Fig. 1: Simulated annealing Fo-Fc omit map for the bound dCTP/3TC-TP in the structure of HIV-1 $RT^{3MB/M184V/F160M}$:DNA.

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References

[1] Y. Yasutake *et al*., *Sci. Rep.***10**, 3021 (2020).
[2] M. T. Miller *et al*., *Protein Sci*. **25**, 46-55 (2015).
[3] S. Nakajima et al., *J. Virol*. in press (2021).

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