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Structures of HIV-1 reverse transcriptase with HBV-associated 3MB/4M mutations complexed with DNA:*E*-CFCP-TP

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1 Introduction

Hepatitis B virus (HBV) polymerase (Pol) is an essential enzyme in the viral replication cycle. However, its three-dimensional structure has not been determined because of its insolubility. Thus, we selected HIV-1 RT carrying HBV-associated amino acid substitutions at the nucleotide-binding site (N-site) as a surrogate for experimental X-ray structural studies of HBV Pol. We have shown that HIV-1 with HBV-associated triple amino acid substitutions 3MB (Q151M/Y115F/F116Y) is highly susceptible to typical anti-HBV nucleoside analogs (NAs), such as entecavir (ETV) and lamivudine (3TC). Furthermore, additional M184V and F160M mutations, known as ETV/3TC-resistant mutations in HBV RT (M204V and L180M in HBV RT), significantly decreased the sensitivity of HIV-1 with RT^{3MB} to ETV and 3TC. The structures of HIV-1 RT^{3MB}, $RT^{3MB/M184V}$ and $RT^{3MB/M184V/F160M}$ provide novel insights into ETV/3TC binding and drug resistance mechanisms that have not been reported in previous *in silico* modeling studies [1, 2].

E-CFCP is a novel anti-HBV guanosine analog with unique exocyclic fluoromethylene and 4'-cyano group that is highly potent against both wild-type and drug-resistant HBV [3]. *E*-CFCP and its triphosphate form (*E*-CFCP-TP) are chemically stable in the human body and exhibit exceptionally long-acting properties, enabling a once-weekly dosing regimen that improves the quality of life of patients with HBV infection. In this study, to obtain a structural basis for the potent antiviral activity of *E*-CFCP, we determined the X-ray structures of HIV-1 RT with HBV-associated mutations 3MB and 4M (3MB + L74V) in complex with a template-primer-mimick DNA aptamer and *E*-CFCP-TP [4].

2 Experiment

HIV-1 RT^{3MB} and RT^{4M} were overexpressed in *Escherichia coli* BL21(DE3)-RIL and purified by Ni-affinity and ion-exchange chromatography, as described previously [1]. A template primer-mimicking 38-mer DNA aptamer [5] was used to prepare the HIV-1 RT:DNA complex. After mixing the RT and DNA, the samples were subjected to gel-filtration chromatography. The HIV-1 $RT^{3MB/4M}$ in complex with DNA were crystallized via hanging-drop vapor-diffusion at 20°C, using a reservoir solution containing 20 mM bis-Tris-HCl pH 6.0, 20-60 mM di-ammonium hydrogen citrate, 20 mM $MgCl_2$, 2-4.5% PEG 6000, 4.8-6.0% glycerol, and 0-2.4% sucrose. Crystals were soaked in a cryoprotectant solution consisting of ~26% glycerol, 12% PEG 6000, and ~5% sucrose supplemented with 2.5 mM *E*-CFCP-TP, and then flash-cooled with liquid nitrogen at 100 K.

3 Results and Discussion

We have determined the structures of HIV-1 RT^{3MB} and RT^{4M} in complex with DNA:*E*-CFCP-TP to a resolution of 2.62 Å and 2.70 Å, respectively. The crystallographic parameters and refinement statistics are listed in Table 1.

Table 1: Data collection and refinement statistics

	RT^{3MB}	RT^{4M}
Ligands	DNA, *E*-CFCP-TP	DNA, *E*-CFCP-TP
Space group	*R*3	*R*3
Unit cell (Å)	a = b = 283.1, c = 95.4	a = b = 284.1, c = 95.6
Resolution (Å)	2.62 (2.67-2.62)	2.70 (2.75-2.70)
R_{meas}	0.074 (0.896)	0.105 (0.878)
I/σ	16.8 (2.1)	10.8 (1.7)
Completeness (%)	99.9 (100.0)	100.0 (100.0)
Multiplicity	5.1 (5.2)	5.5 (5.5)
R_{work}/R_{free}	0.179/0.223	0.173/0.231

The Fo-Fc omission map unambiguously showed the bound *E*-CFCP-TP within the N site (Fig. 1). The heterodimeric p66-p51 structures of RT^{3MB} and RT^{4M} were similar and well-aligned, with a main-chain root mean square deviation (RMSD) of ~1.0 Å. A structural comparison of HIV-1 $RT^{3MB/4M}$ in complex with DNA and *E*-CFCP-TP revealed that the bound *E*-CFCP-TP was

slightly skewed and deviated from the normal binding position of the dNTP (Fig. 2). This deviated binding likely occurred as a result of the protruding fluoromethylene and 4'-cyano groups of *E*-CFCP-TP fitting into the hydrophobic pocket of the N-site. Interestingly, this deviated binding position of *E*-CFCP-TP partly corresponded to that of the bound dCTP observed in HIV-1 RT^{3MB} with the drug-resistant mutations M184V/F160M. Thus, it is likely that this deviated binding mode could evade the structural effects caused by the drug-resistance mutation M184V/F160M.

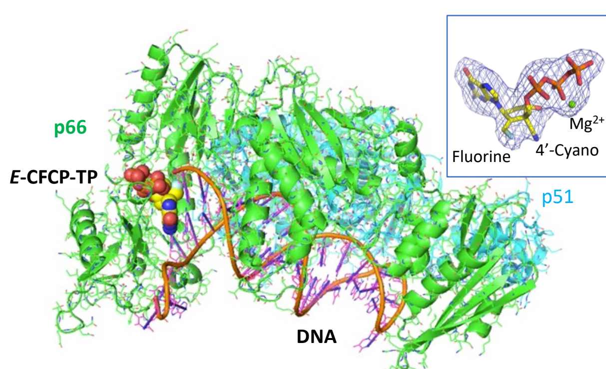

Fig. 1: Overall structure of HIV-1 $RT^{3MB/4M}$ with DNA and *E*-CFCP-TP. Simulated annealing mFo-DFc omit map for the bound *E*-CFCP-TP-Mg^{2+} is also shown.

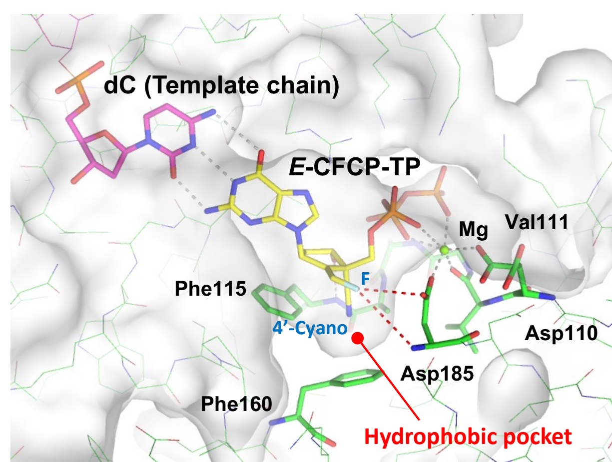

Fig. 2: Overall view for the HIV-1 $RT^{3MB/4M}$ N-site with DNA, *E*-CFCP-TP and Mg^{2+}. Nearby residues are labelled, and the hydrophobic pocket is indicated.

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