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Structure analyses of HIV-1 reverse transcriptase with HBV-associated 4M/4MA/5MB mutations with bound DNA:dGTP

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1 Introduction

Hepatitis B virus (HBV) polymerase (Pol) is a pivotal enzyme for viral replication, while its three-dimensional structure has not been determined because of its unstable and insoluble properties. Therefore, we selected HIV-1 RT with HBV-associated amino acid substitutions at nucleotide-binding site (N-site) as a surrogate for experimental structural studies of HBV Pol. We have previously shown that HIV-1 with the three HBV-associated amino-acid substitutions (3MB: Q151M/Y115F/F116Y) is highly susceptible to the anti-HBV nucleoside analogs (NAs), entecavir (ETV) and lamivudine (3TC). 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. The structures of HIV RT^{3MB}, RT$^{3MB/M184V}$ and RT$^{3MB/M184V/F160M}$ provided insights into the ETV/3TC binding and drug resistant mechanisms [1, 2]. In this study, we newly selected I63/L74 near the nucleotides of the template strand that form base pairs with the NAs/dNTPs, created HIV-1 RT mutants with HBV-associated 3MB/L74V (4M), 3MB/I63V (4MA) and 3MB/L74V/I63V (5MB), and determined their structures in complex with DNA:dGTP.

2 Experiment

HIV-1 RT^{4M}, RT^{4MA} and RT^{5MB} were overproduced using *Escherichia coli* BL21(DE3)-RIL, and purified by Ni-affinity and ion-exchange chromatography as described previously [1, 3]. A template-primer-mimic 38-mer DNA aptamer [3] was used for preparation of RT:DNA complex. After mixing RT and DNA, samples were loaded on gel-filtration chromatography [1]. The HIV-1 RT$^{4M/4MA/5MB}$ in complex with DNA were crystallized by the hanging-drop vapor-diffusion method at 20°C, with a reservoir solution containing bis-Tris-HCl pH 6.0, ammonium di-hydrogen citrate, $MgCl_2$, PEG 6000, glycerol, and sucrose. Crystals were soaked in a cryoprotectant solution consisting of 25% glycerol, 15% PEG 6000, and 15% sucrose supplemented with dGTP, and then flash-cooled in liquid nitrogen at 100 K.

3 Results and Discussion

We have solved the structures of HIV-1 RT^{4M}, RT^{4MA} and RT^{5MB} in complex with DNA:dGTP at resolution of 2.31 Å, 2.22 Å, and 2.24 Å, respectively. These structural data are the highest resolution of any previously reported HIV-1 RT:DNA complex, and provide accurate atomic information on the side chains of all amino acids forming the N-site. The Fo-Fc map clearly showed the bound dGTP-Mg^{2+} within the active site (Fig. 1). The model refinement and the detailed structural comparison with those of previously reported RT^{WT} and RT^{3MB} are in progress.

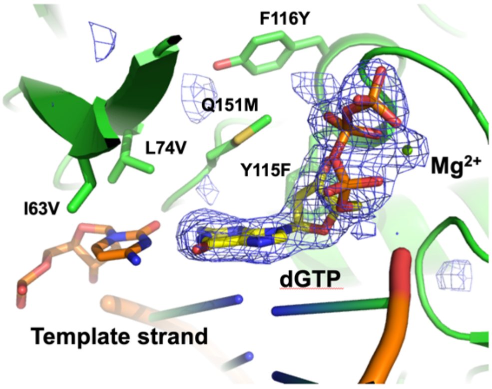

Fig. 1: Unbiased Fo-Fc omit map for the bound dGTP-Mg^{2+} within the N-site structure of HIV-1 RT^{5MB}. The mutated residues are labelled.

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