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# The impact of molecular manipulation in residue 114 of human immunodeficiency virus type-1 reverse transcriptase on dNTP substrate binding and viral replication

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ABSTRACT

Human immunodeficiency virus type-1 (HIV-1) reverse transcriptase (RT) has a unique tight binding to dNTP substrates. Structural modeling of Ala-114 of HIV-1 RT suggests that longer side chains at this residue can reduce the space normally occupied by the sugar moiety of an incoming dNTP. Indeed, mutations at Ala-114 decrease the ability of RT to synthesize DNA at low dNTP concentrations and reduce the dNTP-binding affinity ($K_d$) of RT. However, the $K_d$ values of WT and A114C RT remained equivalent with an acyclic dNTP substrate. Finally, mutant A114 RT HIV-1 vectors displayed a greatly reduced transduction in nondividing human lung fibroblasts (HLFs), while WT HIV-1 vector efficiently transduced both dividing and nondividing HLFs. Together these data support that the A114 residue of HIV-1 RT plays a key mechanistic role in the dNTP binding of HIV-1 RT and the unique viral infectivity of target cell types with low dNTP pools.

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## Introduction

Human immunodeficiency virus type-1 (HIV-1) belongs to the genus lentivirus in the family *Retroviridae*. Most retroviruses in this family, such as alpha, beta, gamma and deltaretroviruses are oncoretroviruses, which exclusively infect dividing cells. Lentiviruses however, productively infect both dividing CD4+ T cells and nondividing cells such as macrophages, which have been shown to act as long-term HIV-1 viral reservoirs (Igarashi et al., 2001; Lewis and Emerman, 1994; Lewis et al., 1992; Weinberg et al., 1991). Due to this dual cellular tropism, the HIV-1 encoded DNA polymerase, reverse transcriptase (RT), must function in two distinct dNTP substrate environments. The first, activated CD4+ T cells, have a cellular dNTP concentration around 1.4–2.5 μM; the second, macrophages, have a much lower concentration of about ~40 nM (Diamond et al., 2004; Kennedy et al., 2010). Our earlier pre-steady-state kinetic analysis reported that HIV-1 RT has a high binding affinity to dNTPs with a $K_d$ near 1 μM, while the $K_d$ value of murine leukemia virus (MuLV) RT was found to be approximately 40 μM (Skasko et al., 2005; Weiss et al., 2004). We also observed that HIV-1 variants harboring RT mutants with a decreased dNTP binding affinity, failed to replicate in macrophages and cell types harboring low dNTP concentrations. However, these mutant RTs do not significantly alter the viral replication in cells containing high dNTP concentrations (Jamburuthugoda et al., 2008; Weiss et al., 2002). Therefore, these studies suggested that the tighter dNTP binding affinity of RT contributes to the unique replication capability of HIV-1 in nondividing cells containing limited dNTP pools.

The DNA polymerase active site of HIV-1 RT contains a series of residues that are responsible for the binding and/or incorporation of dNTPs onto the 3′ end of a primer (Harris et al., 1998). The residues A113, A114, Y115, Q151, and M184 are found near the 3′ OH of an incoming dNTP (Fig. 1) and are all highly conserved in HIV-1 RT as well as other lentiviral RTs (Cases-González and Menéndez-Arias, 2005; Huang et al., 1998; Lowe et al., 1991; Pandey et al., 1996). Y115 (green) appears to play a key role as a gate, discriminating dNTPs from rNTPs (Boyer et al., 2000; Gao et al., 1997; Joyce, 1997; Martín-Hernández et al., 1996). Mutations at residue M184 (purple), which naturally contribute to mismatch selectivity, are frequently found in HIV-1 drug resistant patients (Menéndez-Arias, 2008; Pandey et al., 1996; Ray et al., 2002; Svedhem et al., 2007; Yang et al., 2008; Yoshimura et al., 1999). M184I, for example, is a transient 3TC resistant mutation, ultimately resulting in M184V (Frost et al., 2000; Mulder et al., 2008; Svedhem et al., 2007). It was found that the beta branched side chain of isoleucine sterically blocks the entry of 3TC into the active site of RT (Sarafianos et al., 1999). We have shown that the M184I mutation also alters the binding of RT to dNTPs, raising the $K_d$ to 56 μM, which is similar to the $K_d$ value of MuLV RT (Skasko et al., 2005). The M184I mutation was also found to change the tropism of an HIV-1 vector, preventing it from transducing human macrophages (Jamburuthugoda et al., 2008; Skasko et al., 2005). We observed a similar outcome with Q151N, a non-

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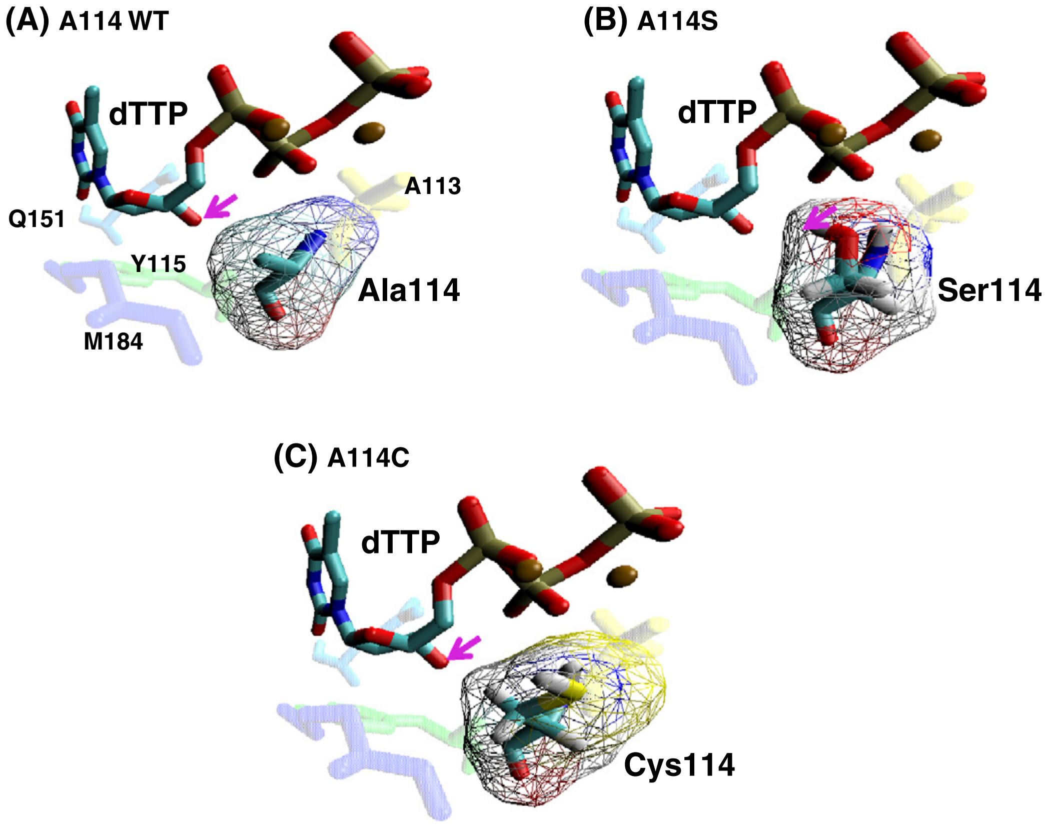

**Fig. 1.** Structural modeling of the HIV-1 RT residue 114 mutants at the dNTP-binding pocket. (A) The position of the HIV-1 RT A114 residue (teal) in relation to an incoming dTTP substrate (blue/red), and Y115 (green), A113 (yellow), M184 (purple) and Q151 (light blue), is shown using coordinate set 1rtd.pdb from the Protein Data Bank (Huang et al., 1998). (B) and (C) The positions of the S114 and C114 mutant residues were constructed by the program PyMOL (Schrodinger, 2010) that manually selected the optimal rotamer for serine or cysteine at residue 114 of HIV-1 reverse transcriptase from Huang et al. (1998). These were then minimized with AMBER 9.0 with 500 cycles (250 steepest descent) with implicit solvent. Arrows indicate the 3′ OH of the incoming dTTP. Volume of amino acids was calculated as described previously (Zamyatnin, 1984).

clinical RT mutation (light blue) (Diamond et al., 2004). The glutamine at 151 interacts with the 3′ OH of an incoming dNTP through a hydrogen bond; however, when mutated to asparagine, the $K_d$ of RT for substrate is raised to 257 μM (Weiss et al., 2002). HIV-1 vectors carrying this mutation also fail to transduce primary human macrophages but maintain a significant level of transduction in cells with high dNTP pools. Principally, this work suggests that the manipulation of certain residues within the active site of RT can impact the dNTP binding affinity through different mechanisms, which result in altered HIV-1 cellular tropism (Diamond et al., 2004).

The A114 residue contributes a nonspecific Van der Waals interaction with the incoming dNTP, unlike Q151, which interacts specifically with the sugar moiety of the dNTP (3′ OH) (Huang et al., 1998; Menéndez-Arias, 2008). The Stanford HIV Database, includes 19 isolates carrying mutations at A114 however, most isolates have been previously shown to be detrimental to RT activity (Halvas et al., 2000; Rhee et al., 2003). A steady-state study demonstrated that a decrease in the $K_m$ values of RT could be correlated with the size of the mutated side chain present at this residue (Cases-González and Menéndez-Arias, 2005). However, the effect of mutations at A114 on the dNTP binding of HIV-1 RT has not been characterized. Structurally, A114 is located in a pocket that binds the 3′ OH of an incoming dNTP, which is in close proximity to the dNTPs sugar moiety (Huang et al., 1998). In this study we hypothesize that mutations at residue 114 of RT to amino acids with larger side chains may reduce the volume of the HIV-1 dNTP-binding pocket, resulting in a decreased space for the sugar moiety of normal dNTPs to reside, but conceivably not dNTP analogs lacking the intact sugar moiety. The requirement of a certain volume in the active site of RT for an incoming dNTP would represent a unique mechanism of substrate binding; unlike the mutation Q151N, that decreases the dNTP binding through a loss of interaction or M184I, which results in a steric clash with the incoming dNTP. We tested this hypothesis to understand the molecular interaction of HIV-1 RT with its dNTP substrate, which plays a key role in HIV-1 proviral DNA synthesis and viral cellular tropism.

## Results

### *Structural view of the wild type and mutant residues at HIV-1 RT position 114*

First, in order to define the position of the methyl side chain of the HIV-1 RT A114 residue with respect to the incoming dNTP, we constructed a model of the dNTP-binding pocket of RT in a ternary complex bound to template/primer and dNTP substrate using a previously determined crystal structure (Huang et al., 1998). As illustrated in Fig. 1A, the methyl side chain of A114 (teal blue) lies near the sugar moiety of the incoming dTTP (light blue/red). More specifically, the methyl side chain of A114 protrudes from the surface of the dNTP-binding pocket toward the sugar moiety of the incoming dNTP, forming a distance of 3.37 Å between the $C^{\beta}$ of A114 and the 3′ OH (pink arrows) of the incoming dNTP (Cases-González and Menéndez-Arias, 2005). This unique geometric position of A114 led us to hypothesize that an increased side chain volume at this residue would conflict with the sugar moiety of an incoming dNTP. Indeed, the reduced space for the sugar moiety of a dNTP appears to be inevitable after calculating the distance of A114S to the 3′ OH, 1.96 Å. However, the distance between the mutated residue and the 3′ OH does not

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