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Review

Molecular mechanisms of HIV-1 resistance to nucleoside reverse transcriptase inhibitors (NRTIs)

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Abstract. Nucleoside reverse transcriptase inhibitors (NRTIs), such as 3′-azido-3′-deoxythymidine, 2′,3′-dideoxyinosine and 2′,3′-dideoxy-3′-thiacytidine, are effective inhibitors of human immunodeficiency type 1 (HIV-1) replication. NRTIs are deoxynucleoside triphosphate analogs, but lack a free 3′-hydroxyl group. Once NRTIs are incorporated into the nascent viral DNA, in reactions catalyzed by HIV-1 reverse transcriptase (RT), further viral DNA synthesis is effectively terminated. NRTIs should therefore represent the ideal antiviral agent. Unfortunately, HIV-1 inevitably develops resistance to these inhibitors, and this resistance correlates with mutations in RT. To date, three phenotypic mechanisms have been identified or proposed to account for HIV-1 RT resistance to NRTIs. These mechanisms include alterations of RT discrimination between NRTIs and the analogous dNTP (direct effects on NRTI binding and/or incorporation), alterations in RT-template/primer interactions, which may influence subsequent NRTI incorporation, and enhanced removal of the chain-terminating residue from the 3′ end of the primer. These different resistance phenotypes seem to correlate with different sets of mutations in RT. This review discusses the relationship between HIV-1 drug resistance genotype and phenotype, in relation to our current knowledge of HIV-1 RT structure.

Key words. Human immunodeficiency virus type 1; reverse transcriptase; nucleoside reverse transcriptase inhibitors; DNA polymerization; chain termination; antiviral drug resistance; phosphorolysis; pyrophosphorolysis.

Introduction

Retroviruses such as the human immunodeficiency virus (HIV) are RNA viruses that replicate through a double-strand DNA intermediate. This novel viral replication cycle requires that retroviruses carry a specific enzyme, reverse transcriptase (RT), since there are no cellular enzymes that can convert single-strand RNA into double-strand DNA. RT is a DNA polymerase that can copy both DNA templates (like cellular enzymes) and RNA templates (unlike cellular enzymes). HIV RT differs from cellular DNA polymerases in two additional respects. First, HIV RT readily utilizes many chemically altered analogs of the normal deoxynucleoside triphosphate (dNTP) DNA polymerase substrates. Second, HIV RT lacks a formal 'proofreading' activity. These characteristic
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NRTIs are analogs of the normal dNTP substrates of DNA polymerases, with important modifications (fig. 1). The 2′,3′-dideoxynucleosides such as ddC and ddI

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lack a 3′-OH on the sugar, whereas other analogs such as 3′-azido-3′-deoxythymidine (AZT) have the 3′-OH replaced by other functional groups that do not allow primer extension. NRTIs require intracellular metabolic transformation for antiviral activity, namely conversion to the triphosphate, a process catalyzed by cellular kinases [2].

After conversion to the active triphosphate, NRTIs must compete with the natural dNTPs both for recognition by RT as a substrate (binding) and for incorporation into the nascent viral DNA chain (catalysis). NRTIs thus inhibit RT-catalyzed proviral DNA synthesis by two mechanisms [3]. First, they are competitive inhibitors for binding and/or catalytic incorporation with respect to the analogous dNTP substrate. Second, they terminate further viral DNA synthesis, due to lack of a 3′-OH group. Chain termination is the principal mechanism of NRTI antiviral action.

NRTIs should be the 'ideal' anti-HIV therapeutics. Each HIV virion carries only two copies of genomic RNA. There are about 20,000 nucleotide incorporation events catalyzed by RT during the synthesis of complete viral DNA, thus providing about 5000 chances for chain termination by any given NRTI. Since HIV-1 RT lacks a formal proofreading activity (i.e. some formal mechanism to identify and excise inappropriate nucleotide incorporation), a single NRTI incorporation event should suffice to quell viral DNA synthesis. In reality, NRTIs are less potent inhibitors of HIV replication than might be expected; reasons for this will be discussed later. In addition, although NRTI therapy is initially quite effective in reducing viral load in HIV-1-infected individuals, the viral burden inevitably rebounds despite continued therapy, due to the appearance of drug-resistant strains of HIV. Numerous mutations in HIV-1 RT have been identified in NRTI-resistant HIV strains (table 1) [4, 5].

The simplest mechanism for resistance would be one of discrimination, i.e. some mechanism for RT to exclude the NRTI, while retaining the ability to recognize the analogous natural dNTP substrate. However, this discrimination is actually somewhat of a problem, since in

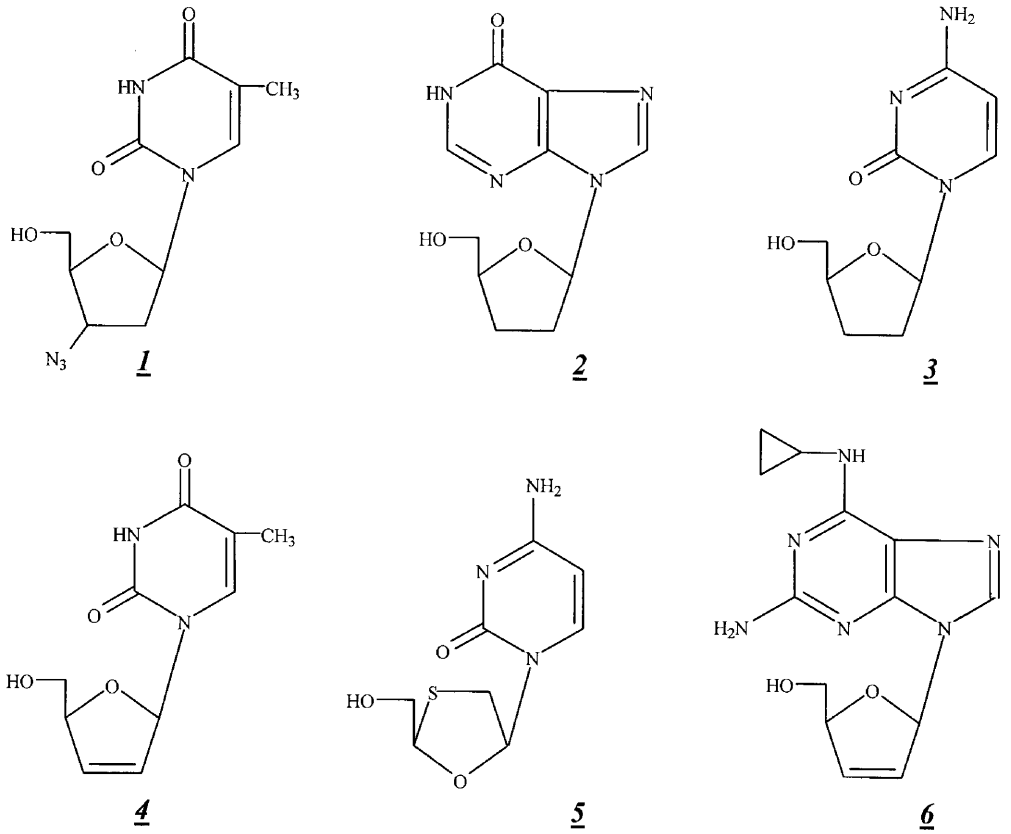

Figure 1. Structures of current clinically-used nucleoside reverse transcriptase inhibitors (NRTI). AZT (1), ddI (2), ddC (3), d4T (4), 3TC (5), abacavir (6).

Table 1. Mutations in HIV-1 RT correlated with resistance to NRTI.

ddN	RT residue											
	41	65	67	69	70	74	75	151*	184	210	215	219
AZT†	M41L		D67N		K70R					L210W	T215Y/F	K219Q
ddC		K65R		T69D			V75T		M184V			
3TC		K65R			K70E				M184V			
ddI (ddA)		K65R				L74V	V75T					
d4T				68↓↓70‡			V75T					
Abacavir		K65R				L74V			M184V			

* The mutation Q151M appears in patients with resistance to multiple ddN.
† High-level AZT resistance requires the presence of two or more mutations.
‡ Two-amino acid insertion mutants are not specific for d4T resistance, but were first identified in patients with d4T resistance. These insertion mutations are found in multidrug resistant HIV-1, generally over a background of AZT-resistance mutations.

Table 2. Resistance phenotypes associated with NRTI-resistance mutations in HIV-1 RT.

Mutation	Resistance conferred	NRTI-resistance phenotype
K65R	ddC, ddI, 3TC, PMEA	discrimination
T69-S-S/G-K70*	multidrug resistance	uncertain (may be combination of discrimination and phosphorolysis)
L74V	ddI, ddC	T/P repositioning
V75T	ddC, d4T	uncertain
E89G	ddG	T/P repositioning
Q151M	multidrug resistance	discrimination
M184I/V	3TC	discrimination (also negative effect on phosphorolysis);T/P repositioning may also play a role
D67N/K70R/T215F(Y)/Q219K	AZT	phosphorolysis
M41L/T215Y	AZT	phosphorolysis†
L210W	AZT	uncertain

* AZT-resistance mutations are required in addition to insertion mutations to provide multi-drug resistance.
† D. Arion, N. Sluis-Cremer, M. A. Parniak, (unpublished).

many cases this requires that RT must selectively ignore a structurally less rich compound (NRTI, lacking the 3′-OH) in favor of the structurally more complex dNTP analog. In addition, our understanding of the molecular aspects of NRTI resistance has been complicated by the complex patterns of mutations required for resistance to some NRTIs such as AZT [6].

To date, three mechanisms have been proposed to account for the molecular basis of the NRTI resistance phenotype. These mechanisms apply to different stages of NRTI inhibition, and include (i) Selective alterations in NRTI binding and/or incorporation (i.e. discrimination), (ii) template/primer (T/P) repositioning, which then influences NRTI incorporation and (iii) phosphorolytic removal of an incorporated chain-terminating NRTI residue from the 3′-end of the nascent viral DNA. Correlations of these phenotypes with specific mutations in RT and the NRTIs affected are summarized in table 2.

HIV-1 RT structure and function

The HIV-1 RT gene encodes a 66-kDa protein; however, the presumed biologically relevant form of HIV-1 RT is a heterodimer comprising of subunits of 66 and 51 kDa (termed p66 and p51) [7]. The p51 subunit is produced during viral assembly and maturation via HIV-1 protease-mediated cleavage of the C-terminal domain of a p66 subunit. The structure of the HIV-1 RT heterodimer is illustrated in fig. 2.

The overall shape of the p66 subunit has been likened to that of a ‘right hand’ [8], with the major subdomains of the polymerase domain of p66 appropriately termed fingers (residues 1–85, 118–155), palm (86–117, 156–237) and thumb (238–318) (fig. 2). The DNA polymerase catalytic aspartate residues (D110, D185, D186) are in the palm subdomain. In addition, the p66 has two additional major subdomains, the ‘connection’ (residues 319–426) and the C-terminal ribonuclease H (RNase

H) (427–565) domains. The latter subdomain is missing in the p51 subunit. Whereas the overall folding of the subdomains is similar in both p66 and p51 subunits, the spatial arrangement of the subdomains differs markedly [8, 9]. The p66 subunit adopts an 'open', catalytically competent conformation that can 'grasp' a nucleic acid template, whereas the p51 subunit is in a 'closed' conformation. The p51 subunit is considered to play a largely structural role, although it may also be important in interacting with the transfer RNA $(tRNA)^{Lys3}$ primer used for the initiation of HIV-1 DNA synthesis [8].

Mutations associated with NRTI resistance occur primarily in the fingers and the palm subdomains of RT (table 1, fig. 2). Because of the nature of HIV-1 RT heterodimer formation, NRTI resistance mutations obviously occur in both p66 and p51 subunits. However, only those in the p66 subunit are generally considered to have phenotypic consequences. Whereas one report has hypothesized that mutations in the p51 subunit may also contribute to the resistance phenotype [10], there are no biochemical data to support this conjecture.

The conversion of HIV-1 genomic RNA into double-strand viral DNA is a complex process, yet all chemical steps are catalyzed by RT. This requires RT to be multifunctional, with two types of DNA polymerase activity, RNA-dependent DNA polymerase (RDDP) to synthesize a DNA strand copy of the viral genomic RNA template and DNA-dependent DNA polymerase

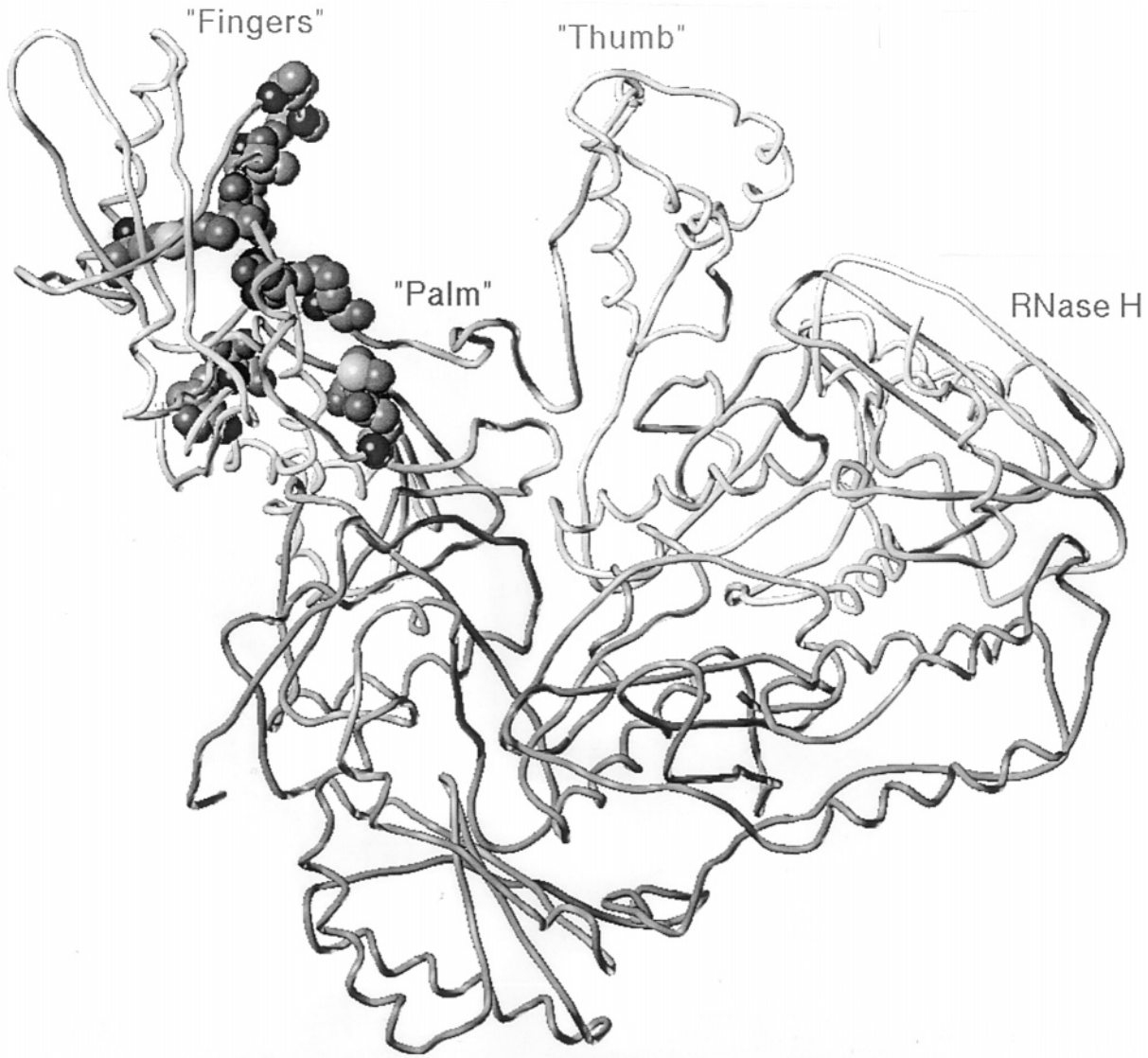

Figure 2. Structure of the HIV-1 RT heterodimer showing locations of residues mutated in NRTI resistance. The crystal coordinates used to generate this figure are Brookhaven Protein Data Bank (PDB) 2HMI [19].

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