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ScienceDirect
Virus Research 134 (2008) 186–202
Virus Research
www.elsevier.com/locate/virusres

Murine leukemia virus reverse transcriptase: Structural comparison with HIV-1 reverse transcriptase

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Available online 21 February 2008

Abstract

Recent X-ray crystal structure determinations of Moloney murine leukemia virus reverse transcriptase (MoMLV RT) have allowed for more accurate structure/function comparisons to HIV-1 RT than were formerly possible. Previous biochemical studies of MoMLV RT in conjunction with knowledge of sequence homologies to HIV-1 RT and overall fold similarities to RTs in general, provided a foundation upon which to build. In addition, numerous crystal structures of the MoMLV RT fingers/palm subdomain had also shed light on one of the critical functions of the enzyme, specifically polymerization. Now in the advent of new structural information, more intricate examination of MoMLV RT in its entirety can be realized, and thus the comparisons with HIV-1 RT may be more critically elucidated. Here, we will review the similarities and differences between MoMLV RT and HIV-1 RT via structural analysis, and propose working models for the MoMLV RT based upon that information.
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Keywords: Reverse transcriptase; RNase H; Moloney murine leukemia virus; Reverse transcription; Retrovirus

1. Introduction

1.1. Reverse transcription

The reverse transcriptases (RTs) are a diverse group of enzymes, however they share two common functions: (1) a DNA polymerization function, which is capable of using either DNA or RNA as a template, and (2) an RNase H function, which serves to hydrolyze the RNA strand within an RNA/DNA hybrid. Both the polymerase and RNase H activities are essential for viral replication.

Reverse transcription is complex and results in the conversion of the viral RNA into dsDNA (for review, see Abbink and Berkhout, 2008; Thomas and Gorelick, 2008). Briefly, reverse transcription initiates from the 3′OH of a cellular tRNA, which is hybridized to the primer-binding site (PBS) within the viral RNA genome. The specific tRNA primer varies between viruses, with Moloney murine leukemia virus (MoMLV) and HIV utilizing $tRNA^{Pro}$ and $tRNA^{Lys,3}$, respectively. RNA-primed-RNA-dependent DNA polymerization elongates the tRNA primer to the terminal 5′ repeat sequence (R) of the RNA, creating the first intermediate, the minus-strand strong-stop DNA ((−)ssDNA). Next, the RNase H must digest the RNA template to allow for the hybridization of the R regions duplicated at the 3′end of the (−)ssDNA and the 3′ end of the genomic RNA, resulting in the first strand transfer. Here the nascent DNA is then elongated via DNA-primed-RNA-dependent polymerization. Again the RNase H is recruited to hydrolyze the RNA template back to the hydrolysis-resistant polypurine tract (PPT), which subsequently serves as the new primer for second-strand DNA synthesis, using RNA-primed-DNA-dependent polymerization to elongate the PPT primer. RNase H is required to remove the PPT and tRNA primers. Removal of the tRNA primer results in the second-strand transfer via the interaction of the complementary PBS sequences. Finally, DNA-primed-DNA-dependent DNA polymerase completes the synthesis of the dsDNA, which retains the LTRs (U3-R-U5) at both of its ends, ready to be processed by the downstream integrase (IN).

1.2. Polymerase subdomains and RNase H

The structure of the *Escherichia coli* Pol I Klenow fragment was found to fundamentally resemble the fingers, palm, and

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doi:10.1016/j.virusres.2008.01.001

thumb of a right hand (Ollis et al., 1985), and this resemblance was extended to the description of the structural subdomains of the RTs. Thus the given subdomain designations in RT are (from N- to C-terminus): fingers, palm, thumb, connection, and the RNase H domain confers multifunctionality to the RT. This anthropomorphic categorizing has turned out to be convenient in making reference to many of the structure/function activities of the RTs.

1.3. RT function

The multifunctional RTs possess two distinct active sites for the complex series of steps needed for proper reverse transcription. First, the polymerase active site, where all DNA elongation steps occur, is comprised of three conserved aspartate residues, and is located on a structurally conserved epitope in the palm subdomain. In addition to the catalytic aspartates, a conserved loop motif, Leu-Pro-Gln-Gly (residues 188–191 in MoMLV RT; residues 149–152 in HIV-1 RT; see Fig. 1), is thought to contribute to the proper positioning of the incoming dNTP with the substrate (Georgiadis et al., 1995). In the HIV-1 RT crystal structure of a ternary complex of RT:template/primer:dNTP, residues from the fingers subdomain, Lys65 and Arg72, are shown to coordinate the triphosphate moiety of the dNTP (Huang et al., 1998). The corresponding residues in MoMLV RT are Lys103 and Arg110, respectively. Second, the RNase H domain, critical for the hydrolysis of the RNA in general as well as for specific cleavages, is located C-terminally and has its active site composed of a conserved Asp-Glu-Asp motif. An additional aspartate residue, which is also conserved among RTs, is located extremely close to the C-terminus of the enzyme, and sometimes is included as part of an essential Asp-Glu-Asp-Asp quartet. RNase H activity has been defined as polymerase-dependent or independent (Furfine and Reardon, 1991), where in the polymerase-dependent mode, the 3′-terminus of the nascent DNA positions the RNase H for cleavage of the RNA template roughly 17–19 nucleotides downstream. Both the polymerase and RNase H active sites require specific divalent cations (Mg^{2+}/Mn^{2+}) for optimal function. These two critical regions in HIV-1 RT and MoMLV RT have distinct similarities as well as characteristic differences.

1.4. HIV-1 RT structure

HIV-1 RT is one of the most extensively studied enzymes in the research community. In addition to the wealth of clinical, biochemical, and biophysical data amassed since the early 1980s, there also have been numerous published X-ray crystal structures of HIV-1 RT. The mature functional form of HIV-1 RT is a heterodimer, comprised of a 66-kDa, 560 amino acid subunit (p66), and a 51-kDa, 440 amino acid subunit (p51). The p66 subunit contains the fingers, palm, thumb, connection, and RNase H. The p51 subunit has the identical sequence of p66, however lacks the RNase H domain. The first structure of HIV-1 RT was in complex with the non-nucleoside reverse transcriptase inhibitor (NNRTI) nevirapine, which in addition to confirming the functional heterodimer, imparted great insight into the flexibility of the subdomains of HIV-1 RT and their functions (Kohlstaedt et al., 1992).

The ensuing multitude of published crystal structures of HIV-1 RT have provided us with probative snapshots of the enzyme as it is working. Several representative structures are those of the unliganded apoenzyme (Esnouf et al., 1995; Rodgers et al., 1995; Hsiou et al., 1996), in complex with various NRTIs (Sarafianos et al., 2002; Tuske et al., 2004), in complex with various NNRTIs (Ding et al., 1995a,b), in complex with dsDNA (Ding et al., 1998), in complex with an RNA/DNA hybrid (Sarafianos et al., 2001), and in a ternary complex with a dsDNA template/primer and incoming dNTP (Huang et al., 1998).

1.5. MoMLV RT structure

Prior to 2004 the only known crystal structures of MoMLV RT were those of the N-terminal fragment, the fingers/palm subdomain. In 1995 the first crystal structure of MoMLV RT fingers/palm was published, making it possible to begin structural comparisons with HIV-1 RT, principally in the polymerase active site region (Georgiadis et al., 1995). Several structures of the MoMLV RT fingers/palm subdomains in complex with various dsDNAs then followed, imparting greater insight into the structural invariance of the region, and detailing a unique (and conserved) binding site for blunt-ended DNA (Coté et al., 2000; Najmudin et al., 2000; Coté and Georgiadis, 2001). Biochemical studies had demonstrated that MoMLV RT was a functional monomer of 671 amino acids, whose subdomains consisted of fingers, palm, thumb, and connection; along with the RNase H domain (Roth et al., 1985; Tanese and Goff, 1988). Absolute structural confirmation of MoMLV RT was achieved with the publication of the crystal structure of the full-length enzyme in complex with duplex DNA (PDB code 1RW3, Das and Georgiadis, 2004). In addition, the crystal structure of the isolated MoMLV RT RNase H domain was recently published (PDB code 2HB5, Lim et al., 2006). Although there are far fewer crystallographic snapshots of MoMLV RT, reasonable postulates as to its structure/function activity may be presented by comparing them with HIV-1 RT.

2. Sequence comparisons of MoMLV RT and HIV-1 RT

It is well known that among the vast array of RTs in the scientific literature the overall sequence identities can be as meager as 10%, however the gross structural folds exhibited by these proteins are quite closely related (for a comprehensive review, see Telesnitsky and Goff, 1997). In some respects it is a difficult task to compare the functional monomeric MoMLV RT with the functional heterodimeric HIV-1 RT, however with the knowledge of the full-length structure of MoMLV RT, the comparisons now can be further elucidated. Using a structure-based sequence alignment (see Fig. 1) in comparing the full length MoMLV RT with HIV-1 RT p66, it readily can be seen that the greatest sequence identity between the two enzymes resides in the fingers/palm subdomains, approaching 25% for the common 243 residues (see Fig. 1). If one allows for very conservative comparisons (e.g., Ala vs. Val, Leu vs. Ile, etc.), then the

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