An Enzymatically Active Chimeric HIV-1 Reverse Transcriptase (RT) with the RNase-H Domain of Murine Leukemia Virus RT Exists as a Monomer

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The existence of retroviral reverse transcriptases as monomers or dimers is rather intriguing. A classical example of the former is murine leukemia virus reverse transcriptase (MuLV RT), while human immunodeficiency virus type 1 (HIV-1) RT represents the latter. A careful scrutiny of the amino acid sequence alignment of the two enzymes pinpoints the region tentatively responsible for this phenomenon. We report here the construction of a chimeric enzyme containing the first 425 amino acid residues from the N-terminal domain of HIV-1 RT and 200 amino acid residues from the C-terminal domain of MuLV RT. The chimeric enzyme exists as a monomer with intact DNA polymerase and RNase-H functions.

Human immunodeficiency virus reverse transcriptase is a heterodimeric enzyme comprised of 66- and 51-kDa subunits. The p51 subunit of the heterodimer is derived from the p66 subunit due to proteolytic cleavage and removal of the RNase-H domain (1–3). Surprisingly, the folding of p51 is remarkably distinct from that of p66. The polymerase domain of p66 folds into an open cleft while that of p51 assumes a closed conformation (4). It has been proposed that the monomeric form of p66 also exists in a closed conformation topologically similar to that of p51 and that the polymerase domain opens up into a large cleft only upon dimerization with p51 (5). The three distinct contact points between p51 and p66 have been discerned from the crystal structure resolution, namely the connection domain, the tip of the finger subdomain of p51, and the extended thumb region that supports the RNase-H region of p66. The asymmetric heterodimer observed in HIV-1 RT is also found in many other retroviruses, namely, FIV, SIV, HIV-2, and EIAV. Interestingly, murine leukemia virus reverse transcriptase is functionally active as a monomer. Although, both MuLV RT and HIV-1 RT exhibit similar enzymatic properties, their architectural organization vastly differs as the former exists as a monomer whereas the latter is a heterodimer. It has not yet been possible to elucidate the region in the sequences of these two reverse transcriptases that necessitates dimer formation in HIV-1 RT while posing no such requirement in MuLV RT. In our effort to identify the region in MuLV RT (which might be missing in HIV-1 RT) that obviates the need for dimerization of this enzyme, we compared the primary sequence of MuLV RT with that of HIV-1 RT. We observed three major motifs containing from 11 to 28 amino acids, which are present in MuLV RT but lacking in HIV-1 RT. We therefore speculated that the conspicuous absence of these motifs in the sequence of HIV-1 RT may have imposed the dimeric requirement for catalytic function. To ascertain this postulation we constructed a chimeric HIV-1 RT containing two of these motifs and the entire RNase-H domain from MuLV RT. The resulting chimeric HIV-1 RT was found to be a monomeric enzyme with intact polymerase and RNase-H functions. These studies form the subject matter of the present investigation.

MATERIALS AND METHODS
DNA restriction enzymes and DNA modifying enzymes, Taq DNA polymerase for polymerase chain reaction (PCR), along with PCR buffer and HPLC-purified dNTPs were purchased from Boehringer Mannheim. Fast flow chelating Sepharose (iminodiacetic-Sepharose) for immobilized metal affinity chromatography was purchased from Amersham Pharmacia Biotech, and ³²P-labeled dNTPs and ATP were the products of NEN Life Science Products. Synthetic oligo-primers were obtained from the Molecular Resource Facility of University of Medicine and Dentistry-New Jersey Medical School and were further purified by polyacrylamide gel electrophoresis (6). All other reagents were of the highest available purity grade and purchased from Fisher, Millipore Corp., Boehringer Mannheim, and Bio-Rad.

Plasmid and Clones—Expression vector pET-24b, pET-28a and Escherichia coli expression strain BL21 (DE3) were obtained from Novagen. The HIV-1 RT, MuLV RT, and Klenow fragment expression clones (pET-28a-RT66, pET-28a-RT51, and pET-28a-MRT) constructed in this laboratory were used for PCR amplification and construction of the chimeric HIV-1 RT (7–10).

Expression and Isolation of Wild Type HIV-1 RT, MuLV RT, and Klenow Fragment—Purification of Moloney murine leukemia virus reverse transcriptase (10) and human immunodeficiency virus type 1 reverse transcriptase (11) and human immunodeficiency virus type 1 reverse transcriptase (11) and Klenow fragment (12) were carried out from recombinant clones according to published protocols. The homodimeric p66/66 HIV-1 RT also contains species of the homodimeric p66/51 generated during the process of purification by bacterial proteolytic cleavage.

Construction and Expression of Chimeric HIV-1 RT—The chimeric HIV-1 RT containing the polymerase domain of HIV-1 RT and the RNase-H domain of MuLV RT was constructed as follows. The 582-base pair sequence containing the entire RNase-H domain of the MuLV RT gene spanning from codons 471 to 670 was amplified by PCR (13). The plasmid pET-28a-MRT containing the coding region for the full-length MuLV RT was used as the template for PCR amplification. The sequences of the up stream primer and the down stream primer were 5′-GTT GTA GCC CTC GTG ACA AAC CCG GCT AGG-3′ and 5′-TAT AGG GCC ATC GAG TAG TAA CAT TAA CCT ATA, respectively. In the same way, we introduced the KpnI restriction site at the 5′-end and XhoI site at the 3′-end of the RNase-H coding sequence of MuLV RT.
Using these restriction sites the amplified fragment was restriction
digested and ligated with the KpnI and Xhol-digested pET-24b-RT51
carrier cassette to construct the pET-24b-CRT\textsubscript{1}, clone. This
carrier cassette contains a 5’ T7 promoter and codes for the chimeric HIV-1 RT
with the metal binding His-Tag sequences at the C-terminal region. The
screening of clones for the appropriate insert (1858 base pairs) was
performed using T7 RNA polymerase. For internal labeling of tRNA\textsubscript{3}Lys—
Amplified by polymerase chain reaction as described (16). The PCR
clone containing the T7 promoter and the gene for full-length tRNA was
obtained from Dr. S. P. J. Le Grice. A 256-base pair region of the
sequence of HIV-1 RT and MuLV RT was
constructed from the bottom and analyzed for their polymerase
function. The reaction products were analyzed by denaturing polyacrylamide-urea gel.

RESULTS AND DISCUSSION

We compared the primary amino acid sequences of HIV-1 RT
and MuLV RT in order to locate the region that confers dimeric or
monomeric structure to these enzymes, respectively. Three
major motifs, A, B, and C in the primary amino acid sequence
of MuLV RT, conspicuously absent in the sequence of HIV-1 RT
emerged (Fig. 1). Motif A, the longest, is comprised of 27 amino
acids spanning residues 479–505 and lies in the putative
connection subdomain of MuLV RT. Motif B, found in the RNase-H
domain spans residues 597–611, while the motif C containing
residues 318–328 is located in the polymerase domain. We
suspected that the absence of these motifs in the primary
amino acid sequence of HIV-1 RT may have imposed the need
for its dimerization. We, therefore, constructed a chimeric
HIV-1 RT containing the first 425 codons from the N-terminal
amino acid sequence of HIV-1 RT and 200 codons from the C-terminal of MuLV RT.
The C-terminal fragment of MuLV RT contained part of its
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HIV-1 RT as a monomer as it sediments at a position higher than the sedimentation position of the dimeric HIV-1 RT but closer to that of the monomeric MuLV RT. Binding studies with tRNA$^{\text{Lys}}$, a natural primer for HIV-1 RT, using gel retardation assay indicated a mobility shift distinct from the wild type HIV-1 RT on native polyacrylamide gel electrophoresis (Fig. 2D). The chimeric HIV-1 RT migrated ahead of the wild type HIV-1 RT, but closer to MuLV RT. This also indicated that, unlike HIV-1 RT, the chimeric RT is a monomeric enzyme. The bands shown in the autoradiogram appear slightly elongated due to the overloading of the tRNA bound enzyme protein on the gel. This was essential in order to obtain sufficient signal as all the three enzymes exhibit poor binding affinity with the synthetic tRNA$^{\text{Lys}}$. HPLC gel filtration of HIV-1 RT, chimeric HIV-1 RT, and MuLV RT (19) using two BioSeph 300 columns (300 × 7.8 mm; Phenomenex Inc.) also indicated the existence of the chimeric enzyme as a monomer (data not shown).

Although MuLV RT exists as a monomer in solution, it has been suggested to be in a dimeric form when bound to the template primer (20). It was therefore interesting to examine if the chimeric HIV-1 RT also dimerizes upon binding to the template primer. We therefore photocross-linked wild type HIV-1 RT, MuLV RT, Klenow fragment, and chimeric HIV-1 RT with 5'-32P-labeled 37-mer self-annealing template primer. The radioactive E-TP covalent species were purified on DEAE-cellulose column and subjected to glycerol gradient ultracentrifugation analysis. The sedimentation profile of labeled E-TP complex was determined by analyzing the gradient fractions by SDS-polyacrylamide gel electrophoresis followed by autoradiography. The results are as shown in Fig. 3 (also see Table I). Contrary to an earlier report (20), the DNA-bound MuLV RT sedimented as a monomer close to the DNA-bound Klenow fragment but farther from the DNA bound dimeric HIV-1 RT. The DNA-bound chimeric HIV-1 RT also sedimented as a monomer at the position between that of the DNA-bound Klenow fragment and MuLV RT. These results provide further proof that both MuLV RT and chimeric HIV-1 RT do not dimerize upon binding to the template primer.

**Fig. 2.** A, SDS-PAGE of the chimeric HIV-1 RT containing the RNase-H domain of MuLV RT. Purified chimeric enzyme was expressed in E. coli BL 21(DE3) and purified by one-step purification through immobilized metal affinity column chromatography and analyzed by SDS-polyacrylamide gel electrophoresis. Lane 1, chimeric HIV-1 RT; lane 2, wild type HIV-1 RT; lane 3, wild type MuLV RT. B, glycerol gradient sedimentation profile of the polymerase activities of the wild type HIV-1 RT, MuLV RT, and chimeric HIV-1 RT. The purified chimeric HIV-1 RT as well as the wild type HIV-1 RT and MuLV RT were individually resolved by 10–30% linear glycerol gradient ultracentrifugation at 48,000 rpm in SW50.1 rotor for 22 h. Gradients were fractionated from the bottom and assayed for polymerase activity. C, SDS-PAGE analysis of the gradient fractions. An aliquot of each gradient fraction was subjected to SDS-polyacrylamide gel electrophoresis, and the protein peak in the fraction was visualized by Coomassie Blue staining of the gel. D, mobility shift assay of tRNA-bound chimeric HIV-1 RT and wild type RTs by native polyacrylamide gel electrophoresis. Lane 1, tRNA$^{\text{Lys}}$; lane 2, wild type HIV-1 RT; lane 3, wild type MuLV RT. The complexes were subjected to gel retardation analysis by polyacrylamide gel electrophoresis on a 7% nondenaturing native gel. The electrophoresis was carried out at in a Tris borate buffer system at 4 °C at 100 volts for 4 h followed by autoradiography.

**Fig. 3.** Glycerol gradient ultracentrifugation analysis of DNA-bound HIV-1 RT, MuLV RT, chimeric HIV-1 RT, and Klenow fragment. Two-hundred microgram of individual enzyme protein was incubated on ice for 10 min with 5'-32P-labeled self-annealing 37-mer TP containing photoactivatable bromodeoxyuridine base at the penultimate nucleotide from the 3' primer terminus (Table I). The mixture was UV-irradiated at 312 nm UV for 3 min in a Spectrolinker (9). The enzyme-TP covalent complex was applied on a DEAE-cellulose column (1 ml) pre-equilibrated with 100 mM NaCl in 50 mM Tris-HCl, pH 7.8. After washing the column with the same buffer (10 ml), the E-TP covalent complex was eluted at 400 mM salt concentration. The uncross-linked DNA remained bound in the column and eluted at 600 mM salt concentration. The purified E-TP covalent complex was then subjected to glycerol gradient analysis as described under “Materials and Methods.” The sedimentation profile of DNA cross-linked enzyme was determined by analyzing every alternate gradient fraction by SDS-PAGE followed by autoradiography.

**TABLE I**

| Sequence of synthetic template and primer oligonucleotides |
|------------------------------------------------------------|
| **Template/primer** |
| 37-Mer self-annealing template primer with photocross-linker  |
| 5'-TCACGTCAAGAAGATCCTCCCTC-3' |
| 18-Mer PBS primer |
| 5'-AAAAGGTGTGACTGATTTTCCC-3' |
| 22-Mer PBS primer |
| 5'-GTCCCCGGTTGGGGGGGCGCCACCAGTC-3' |
| 49-Mer U5-PBS DNA template |
| 3'-CAGGGCAGCAGCGCGCGGTTGAGCTTCTCT |
| 30-Mer U5-PBS RNA |
| 3'-AAAAGGTTGAGCTTTCCCTCC-5' |
The purified chimeric HIV-1 RT was examined for its DNA polymerase activity employing homopolymeric and heteropolymeric RNA and DNA templates primed with 5'-32P-labeled DNA primers (7). The chimeric enzyme was found to catalyze DNA synthesis efficiently on both RNA and DNA templates, yielding RNA:DNA hybrid and duplex DNA, respectively (Fig. 4). The cleavage product of the RNA strand was analyzed on a denaturing polyacrylamide-urea gel.

The RNase-H activity of the chimeric HIV-1 RT was examined for its DNA polymerase activity employing homopolymeric and heteropolymeric RNA and DNA templates primed with 5'-32P-labeled DNA primers (7). The chimeric enzyme was found to catalyze DNA synthesis efficiently on both RNA and DNA templates, yielding RNA:DNA hybrid and duplex DNA, respectively (Fig. 4). As shown in the figure, the extent of full-length product catalyzed by the chimeric enzyme was similar to that found with wild type HIV-1 RT and MuLV RT. Further analysis shown in Fig. 5 demonstrates the ability of the chimeric enzyme to catalyze both endonucleolytic and processive RNase-H functions in a manner similar to the wild type HIV-1 RT and MuLV RT.

The region responsible for conferring monomeric structure to the chimeric HIV-1 RT seems to be the introduction of either motif A or motif B contributed by nonconserved connection subdomains and RNase-H domain of MuLV RT. Interestingly, Motif A region of MuLV RT exhibits 46% similarity and 21% identity with the region spanning residues 108–141 in the N-terminal region of HIV-1 RT. This region of HIV-1 RT contains β6, αC, αD, β7, and β7–β8 loops, which are constituents of the finger and palm subdomains of the enzyme. The β7 and β7–β8 loop in p66 are far away from the catalytic cleft, while in the p51 subunit they are in the vicinity of the catalytic cleft of the p66 subunit (23). In p51, the β7–β8 loop also contains the interaction site for a non-nucleoside inhibitor (Glu-138), TSAO. Interaction of Glu-138 of the p51 subunit with TSAO results in delayed dissociation of p66/p51 heterodimeric HIV-1 RT. DNA binding ability of TSAO-treated HIV-1 RT and chimeric HIV-1 RT. Three micrograms of enzyme protein pretreated with 5-fold molar excess of non-nucleoside inhibitor, TSAO, was incubated on ice with 5'-32P-labeled 37-mer self-annealing template primer for 5 min. The mixture was UV-irradiated at 312 nm for 3 min in a Spectrolinker, and the E-TP covalent complex was resolved by SDS-PAGE (9). The elution buffer contained 150 mM NaCl in 50 mM Tris-HCl, pH 8.0 (19). The elution time of dissociated p66 and p51 monomers was significantly delayed by 0.67 and 1.4 min, respectively, as compared with the p66/p51 heterodimeric HIV-1 RT. B. DNA binding ability of TSAO-treated HIV-1 RT and chimeric HIV-1 RT. Three micrograms of enzyme protein pretreated with 5-fold molar excess of non-nucleoside inhibitor, TSAO, was incubated on ice with 5'-32P-labeled 37-mer self-annealing template primer for 5 min. The mixture was UV-irradiated at 312 nm for 3 min in a Spectrolinker, and the E-TP covalent complex was resolved by SDS-PAGE (9). C. Effect of TSAO on the polymerase activity of wild type HIV-1 RT, MuLV RT, and chimeric HIV-1 RT. Poly(rA)5'-32P-labeled (dT)18 homopolymeric TP was used to assess the polymerase activity of TSAO treated (+) and untreated (−) enzymes under standard reaction conditions. The products were analyzed on a denaturing polyacrylamide-urea gel.

MuLV RT. This is so far the first report that a chimeric HIV-1 RT containing the entire RNase-H domain from MuLV RT has been constructed with intact RNase-H and DNA polymerase functions. Earlier Hizi et al. (21) constructed two chimeric HIV-1 RTs and found them both to be inactive with respect to their polymerase activities while exhibiting low levels of RNase-H activity. Post et al. (22) have constructed a chimeric RT by replacing the RNase-H domain of MuLV RT with E. coli RNase-H. The resulting enzyme was found to be impaired in catalyzing DNA synthesis, although it exhibited 300-fold higher RNase-H activity as compared with the wild type MuLV RT. It is not known whether chimeric MuLV RT with intact RNase-H domain can be constructed.
zyme the binding site for TSAO may not exist. We propose that absence of the subunit interacting surface in the chimeric enzyme and polymerase activity of the TSAO-treated chimeric enzyme remained unaffected (Fig. 6, A–C). Interestingly, the chimeric HIV-1 RT is completely insensitive to TSAO treatment, as both the DNA binding ability and polymerase function (Fig. 6, B and C). Unlike, in the wild type HIV-1 RT, the β7β8 loop in the chimeric HIV-1 RT may be inaccessible for binding to the TSAO, thus manifesting in a complete resistance to this inhibitor. Alternatively, due to the absence of the subunit interacting surface in the chimeric enzyme the binding site for TSAO may not exist. We propose that the β7-β8 loop of the p51 subunit of HIV-1 RT may structurally compensate for the missing motif A in the connection subdomain of HIV-1 RT. Insertion of this motif in the connection subdomain of HIV-1 RT is currently being carried out to ascertain whether the resulting enzyme is functional in the monomeric form.

Why does the p66 subunit of HIV-1 RT need leverage from the p51 subunit? Can it support its open conformation independent of p51? One possibility is that the elongated p66 subunit may be unable to hold its polymerase and RNase-H domains in an unsteady horizontal position and therefore may require support from p51. In the chimeric RT, the positioning of the polymerase and RNase-H domains may be such that one domain supports the other thus circumventing the need for its dimerization. Probably, the extended connection subdomain may help in positioning both the domains in such a fashion. This presumption is supported by the observation that the putative connection subdomain of MuLV RT, as determined by aligning of its primary amino acid sequence with that of HIV-1 RT, is longer than that of HIV-1 RT, and this may possibly be one of the factors for its monomeric structure.

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REFERENCES
1. Di Marzo Veronese, F., Copeland, T. D., DeVico, A. L., Rahman, R., Oroszlan, G., Gallo, R. C., and Sarangadharan, M. G. (1986) Science 231, 1289–1291
2. Wondrak, E. M., Lower, J., and Kurth, R. J. (1986) Gen. Virol. 67, 2791–2797
3. Lightfoot, M. M., Coligan, J. E., Folks, T. M., Fauci, A. S., Martin, M. A., and Venketesan, S. (1986) J. Virol. 60, 771–775
4. Kohlstaedt, L. A., Wang, J., Friedman, J. M., Rice, P. A., and Steitz, T. A. (1992) Science 256, 1783–1789
5. Wang, J., Smerdon, S. J., Jager, J., Kohlstaedt, L. A., Rice, P. A., Friedman, J. M. (1994) Proc. Natl. Acad. Sci. U. S. A. 91, 7242–7246
6. Maxam, A., and Gilbert, W. (1980) Methods Enzymol. 65, 499–560
7. Pandey, V. N., Kaushik, N., Rege, N., Sarafianos, S. G., Yadav, P. N. S., and Modak, M. J. (1996) Biochemistry 35, 2168–2179
8. Kaushik, N., Rege, N., Sarafianos, S. G., Yadav, P. N. S., Modak, M. J., and Pandey, V. N. (1996) Biochemistry 35, 11536–11546
9. Kaushik, N., Harris, D., Rege, N., Modak, M. J., Yadav, P. N. S., and Pandey, V. N. (1997) Biochemistry 36, 14430–14438
10. Chowdhury, K., Pandey, V. N., and Modak, M. J. (1996) Biochemistry 35, 16610–16629
11. Lee, R., Kaushik, N., Modak, M. J., Vinayak, R., and Pandey, V. N. (1998) Biochemistry 37, 900–910
12. Kaushik, N., Pandey, V. N., and Modak, M. J. (1966) Biochemistry 35, 7256–7266
13. Saiki, R. K., Scharf, S. J., Faloona, Mullis, K. B., Horn, G. T., Ehrlich, H. A., and Arnheim, N. (1985) Science 230, 1350–1354
14. Sarafianos, S. G., Pandey, V. N., Kaushik, N., and Modak, M. J. (1995) J. Biol. Chem. 270, 19729–19735
15. Richter, N. J., Howard, K. J., Cirino, N. M., Wohrl, B. M., and Le Grice, S. F. J. (1992) J. Biol. Chem. 267, 15952–15957
16. Sanger, F., Nicklen, S., and Coulson, A. R. (1977) Proc. Natl. Acad. Sci. U. S. A. 74, 5463–5467
17. Modak, M. J., and Marcus, S. L. (1977) J. Biol. Chem. 252, 11–19
18. Modak, M. J., and Wohrl, B. M. (1990) Virology 175, 575–580
19. Post, K., Guo, J., Kalman, E., Uchida, T., Crouch, R. J., Levin, J. G. (1993) Biochemistry 32, 5508–5517
20. Jacobs-Molina, A., Ding, J., Nanni, R. G., Clark, A. D., Lu, X., Tantillo, C., Williams, R. L., Kamer, G., Ferris, A. L., Clark, P., Hizi, A., Hughes, S. H., and Arnold, E. (1993) Proc. Natl. Acad. Sci. U. S. A. 90, 6320–6324
21. Jonckheere, H. Baymaans, J. M., Balzarini, J., Velasquez, S., Camarasa, M. J., Desmyter, J., De Clercq, E., and Anne, H. (1994) J. Biol. Chem. 269, 25255–25258
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