Reconstitution and Properties of Homologous and Chimeric HIV-1·HIV-2 p66·p51 Reverse Transcriptase*

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Metal chelate affinity chromatography has been used to follow reconstitution of the 66- and 51-kDa human immunodeficiency viruses (HIV-1 and HIV-2) reverse transcriptase (RT) subunits into heterodimer, as well as chimeric enzymes comprised of heterologous subunits. By adding a small N-terminal polyhistidine extension to the 51-kDa subunit of either enzyme, reconstituted RT could be recovered from a cell lysate by chromatography on Ni²⁺-nitrilotriacetic acid-Sepharose. Homologous RT subunits rapidly associated to form the respective heterodimers (1-p66·1-p51 and 2-p66·2-p51) when bacterial lysates containing the individual components were mixed. Under the same conditions, association of p66 HIV-2 and p51 HIV-1 RT was inefficient and could be improved slightly by prolonged incubation of the respective p66 and p51 subunits. In contrast, HIV-1 p66 RT rapidly associated with the 51-kDa subunit of the HIV-2 enzyme. RNA-dependent DNA polymerase activity was associated with all reconstituted enzymes, and the response of each chimeric RT to an inhibitor selective for the HIV-1 enzyme indicated that sensitivity to inhibition was determined by the source of its 66-kDa subunit.

The multifunctional reverse transcriptase (RT) of the types 1 and 2 human immunodeficiency viruses (HIV-1 and HIV-2) remains an attractive candidate for antiviral therapy to stem the progression of acquired immune deficiency syndrome (AIDS). In addition to RNA- and DNA-dependent DNA polymerase activities (1, 2), its ribonuclease H (RNase H) function (3) has recently been demonstrated to be a potential target for therapeutic intervention (4–6). Huber and Richardson (8) have recently shown that HIV-1 p66 RT specifically selects the polypurine tract primer (7) generated by its inherent RNase H activity, for initiation of (+)-strand synthesis, proposing this replication step as a target for antiviral drugs. The affinity of the HIV-1 enzyme for both synthetic and natural forms of its cognate replication primer, tRNA,3G (9, 10), coupled with the ability of tRNA to inhibit cDNA synthesis from a synthetic template-primer (11) suggests that disruption of the specific RT·tRNA complex prior to initiation of (+)-strand DNA synthesis may be possible (6). Finally, Restle et al. (12) have presented data suggesting that monomeric forms of the HIV-1 RT (p66 and p51) are enzymatically inert. These workers observed significant activity in heterodimeric RT as well as either form of homodimer (p66·p66 and p51·p51), suggesting a novel therapeutic approach of designing agents that act at the dimer interface and prevent subunit association. A better understanding of factors governing dimer formation would facilitate this approach. In this communication, we have used a metal chelate affinity resin, Ni²⁺-NTA-Sepharose (13), to monitor association of recombinant HIV-1 and HIV-2 RT subunits into either homologous or chimeric heterodimers and analyzed the response of the partially purified enzymes to an RT inhibitor selective for the HIV-1 enzyme. Although site-directed mutagenesis has been employed by Larder et al. (14) to define active site residues of HIV-1 RT, lack of structural data on this enzyme makes an equivalent analysis of residues involved in dimerization difficult. Chimeric HIV/murine leukemia virus RT. RNase H have recently been prepared to help understand the relationship between the polymerizing and RNase H domains of these enzymes (14). However, since the boundaries of the several RT domains (e.g. substrate, template and tRNA primer binding sites) are not accurately defined, the results obtained from fusion of portions of the HIV-1 and -2 enzymes might be difficult to interpret. In light of this, we have sought an alternative approach to assess factors contributing to HIV RT subunit association.

Recently, we prepared a selectively deuterated HIV-1 RT (p66/D·p51) for neutron solution scattering analysis. This involved reconstitution from recombinant Escherichia coli strains expressing the individual subunits. Purification of the reconstituted enzyme was aided by a small polystyrene extension added selectively to the N terminus of the recombinant 51-kDa subunit. Reconstituted heterodimer RT bearing the polystyrene extension solely on its 51-kDa subunit could be purified free of non-heterodimer-associated p66 by metal chelate affinity chromatography (13, 16). This approach is analogous to previous work for which we used an expression cassette, pRT6H-PROT, to prepare heterodimer in which the 66-kDa subunit selectively bore a polystyrene extension (16). Based on these observations, we reasoned that a similar strategy might be employed to study not only reconstitution of homologous heterodimers but also the proficiency with which subunits from the HIV-1 and HIV-2 enzymes associate. The precedent for these studies lay partly in a recent report that a tetrahydroimidazo[4,5,1-jk][1,4]benzodiazepin-2(1H)-one (TIBO) derivative, R82150, selectively inhibits the HIV-1 enzyme (17). Similar selectivity for HIV-1 RT has been demonstrated with the non-nucleoside 6,11-dihydro-11-cyclopropy1-4-methylpyrido[2,3-b:2',3'-e][1,4]diazepin-6-one

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The abbreviations used are: RT, reverse transcriptase; HIV, human immunodeficiency virus; NTA, nitrilotriacetic acid; TIBO, tetrahydroimidazo[4,5,1-jk][1,4]benzodiazepin-2(1H)-one.  

1 H. Lederer, D. Schatz, R. May, H. Crespi, J.-L. Darlix, S. Le Grice, and H. Heumann, manuscript in preparation.
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(BI-RG-587) (18, 19). These results indicate subtle differences between the HIV-1 and HIV-2 enzymes, despite approximately 60% amino acid homology, which underscore the importance of understanding the nature of each enzyme when attempting to develop a common and effective inhibitor.

In the present communication, we present data indicating that the N-terminal polyhistidine-extended form of HIV-1 or HIV-2 p51 rapidly reconstitutes with its homologous p66 counterpart, yielding an enzymatically active HIV-1 (1-p66-1-p51) or HIV-2 (2-p66-2-p51) heterodimer. When the same strategy is employed to reconstitute a chimeric heterodimer of p66 HIV-2 and p51 HIV-1 RT, virtually all the 66-kDa HIV-2 subunit is recovered in the nonbinding faction, whereas the polyhistidine-extended HIV-1 p51 is retained by the column. Prolonged incubation of heterologous subunits prior to metal chelate chromatography results in limited reconstitution of a chimeric heterodimer. In contrast, HIV-1 p66 RT is proficient in associating with the HIV-2 51-kDa polypeptide. These combined observations indicate subtle differences in the domains of the enzymes involved in dimer formation.

Finally, the response of homologous and chimeric heterodimer RT preparations to the inhibitor R82150 (17), specific for the HIV-1 enzyme was assayed. In accordance with recent data, p66-p51 HIV-1 RT was sensitive, and p66-p51 HIV-2 RT was resistant to inhibition. The response of chimeric heterodimers to inhibition by R82150 indicates that the sensitivity to inhibition is determined by the nature of the 66-kDa subunit.

EXPERIMENTAL PROCEDURES

Reverse Transcriptase Plasmids

E. coli strains independently expressing the 51- and 66-kDa forms of both HIV-1 and HIV-2 RT were used in the reconstitution experiments. HIV-1 subunits were expressed from plasmids pRT (p66) and p6HRT51 (His6-p51), the latter introducing a (His)6 extension on the N terminus of the RT subunit. With this extension, reconstituted heterodimer could be recovered from a combined cell homogenate by metal chelate affinity chromatography on Ni2+-NTA-Sepharose (13). A similar HIV-2 p51 RT construct, derived from the HIV-2moc isolate (20) by the polymerase chain reaction (21), was expressed from the plasmid pH2RT51. In order to produce a non-histidine extended form of HIV-2 p66, a twin HIV-2 RT-HIV-1 protease cassette, p2RT-PROT, was constructed in a manner similar to our previously reported HIV-1 heterodimer-producing strains pH6RT-PROT and pH6TH-PROT (16). The HIV-2 RT component of the cassette differed inasmuch as it was preceded by 8 amino acids of HIV-2 protease (Bao61 cleavage site) and followed by 44 amino acids of integrase (HinCII cleavage site). This approach was adopted to prepare HIV-2 RT with N- and C-terminal sequences consistent with the viral enzyme in a manner similar to that reported by Mizrahi et al. (22) for HIV-1 RT. The present communication demonstrates that maturation of this slightly elongated RT polypeptide by HIV-1 protease yields correctly sized, enzymatically active p66 HIV-2 RT. As was observed with our homologous (23) or chimeric (24) HIV-2 p66 clones, p51 HIV-2 RT was not recovered from the twin expression cassette contained on plasmid p2RT-PROT. The expression cassettes from which homologous and chimeric heterodimer RT were prepared have been summarized in Fig. 1A.

Microbiological Manipulations

All RT-producing plasmids were maintained under lac-inducible control in the E. coli strain pDM1. In the presence of 100 µg/ml ampicillin and 25 µg/ml kanamycin (25). Induction of gene expression was achieved by growing the appropriate strains in antibiotic-supplemented L-broth until A600,000 = 0.7, followed by addition of isopropyl-β-D-thiogalactopyranoside to a final concentration of 200 µg/ml. RT expression was allowed to proceed for an additional 4 h, after which cultures were harvested. Bacterial pellets were stored at −20°C prior to reconstitution experiments. 500-ml isopropyl-β-D-thiogalactopyranoside-induced cultures of the appropriate strains were used in all reconstitution experiments.

RT Reconstitution

HIV-1 and HIV-2 Heterodimers and Chimeric HIV-1 p66-HIV-2 p51−p66 HIV-1 RT was prepared by combining pellets (≈1.5 g each) of cultures producing (His6)-p51 and p66; in an analogous manner, p66-p51 HIV-2 RT was prepared by combining pellets containing (His)6-p51 and p66 subunits. Pellets were disrupted by lysozyme treatment and sonication as previously described (16). Following ultracentrifugation (35,000 rpm, 30 min, 4°C), the supernatant was applied directly to a 4-ml column of Ni2+-NTA-Sepharose, previously equilibrated in Buffer A/78 (50 mM NaH2PO4/Na2HPO4, pH 7.8, 0.5 mM NaCl). Following sample application, the column was extensively washed in Buffer A/78 (~5 column volumes), after which weakly adsorbed proteins were eluted in 10 volumes of Buffer A/60 (50 mM NaH2PO4/Na2HPO4, pH 6.0, 0.3 mM NaCl, 10% glycerol). RT was eluted by application of a 25-ml linear gradient of 0-0.5 M imidazole in Buffer A/60. Elution from the column was monitored initially by A280. Subsequently, samples indicated in the text were analyzed for the presence of immunoreactive RT polypeptides. The same approach was adopted for the preparations of chimeric heterodimer 1-p66-2-p51.

HIV-2 RT-2p66-HIV-1 p51 RT—The protocol for heterodimer reconstitution was modified to prepare the chimeric HIV-2p66-HIV-1p51 enzyme. Initially, the reconstitution protocol described in the previous section was followed. This procedure resulted in HIV-2 p66 RT in the nonbinding faction and HIV-1 p51 in the 0-0.5 M imidazole eluate. Samples containing HIV-2 p66 were pooled, dialyzed overnight in 50 mM Tris/HCl, pH 7.5, and filtered through a 0.2-µm filter. Mixed enzyme in Buffer A/78, 5% (v/v) glycerol to reduce the NaCl concentration, and then applied to a 4-m1 column of DEAE-Sepharose (Pharmacia LKB Biotechnology Inc.) equilibrated with the same buffer. Under these conditions, partially purified p66 HIV-2 RT was recovered in the nonbinding fraction. DEAE-Sepharose-purified HIV-2 p66 RT was then mixed with HIV-1 p51 eluted from Ni2+-NTA-Sepharose (previously dialyzed against Buffer A/78 to remove imidazole). Mixed subunits were allowed to incubate 12 h at 4°C before being reapplied to Ni2+-NTA-Sepharose. After washing with Buffer A/78, RT was eluted with a gradient of 0-0.5 M imidazole in Buffer A/78.

Immunological Analyses

Following induction of protein synthesis, 1-ml culture portions were centrifuged and the pellets resuspended in 200 µl of 50 mM sodium dodecyl sulfate-polyacrylamide gel electrophoresis sample buffer (25). Samples from Ni2+-NTA-Sepharose were diluted 1:1 with 2 x gel sample buffer. After heating at 100°C for 10 min, 10-µl aliquots were loaded onto 10% sodium dodecyl sulfate-polyacrylamide gels containing a 3.3% stacking gel. Following electrophoresis, fractionated proteins were transferred to nylon membranes (Immobilon P, Millipore) according to the method of Towbin et al. (27). Since antiserum against the HIV-2 enzyme was not available, all samples were immunologically with rabbit polyclonal antiserum raised against purified recombinant HIV-1 RT. Detection of immunoreactive polypeptides was accomplished using an alkaline phosphatase-coupled second antibody (Bio-Rad). HIV-2 RT subunits were observed to cross-react with antibodies against the HIV-1 enzyme, although to a slightly lesser extent.

Reverse Transcriptase Assays

The activities of all RT preparations were determined by incorporation of dGTP into polynucleotide from a poly(rC)-oligo(dG) template-primer. Reaction mixtures (70-µl) were comprised of 50 mM Tris/HCl, pH 8.0, 100 mM KCI, 6 mM MgCl2, 1 mM dithiothreitol, 50 µg/ml bovine serum albumin, 10 µg/ml poly(rC)-oligo(dG) (7:3 mass ratio), and 10 µM [γ-32P]dGTP (3000 cpm/pmol, Du Pont-New England Nuclear). Reaction mixtures were preincubated at 37°C, followed by addition of RT. Mixtures were incubated 20 min at 37°C and trichloroacetic acid-insoluble radioactivity collected on GF/C filters (Whatman). Incorporation was quantified by liquid scintillation counting. 1 unit of HIV RT activity is defined as the amount catalyzing incorporation of 1 pmol of dGTP into polynucleotide in 20 min under the above mentioned conditions.

The B20 derivative R82150 (17), synthesized in the Department of Anti-infective Chemistry, Roche Research Center, Nutley, NJ, was dissolved in dimethyl sulfoxide to a final concentration of 10 mM and stored at −20°C. This stock was diluted immediately prior to use in
enzyme assays. Assay conditions for analysis of inhibition by R82150 were as described above, and the amount of RT added was adjusted to support incorporation of ~7 pmol of dGTP into polynucleotide in 20 min.

RESULTS

Experimental Strategy—Fig. 1A indicates the strategy we have employed for reconstitution of homologous HIV-1 and HIV-2 heterodimers as well as chimeric HIV-1-HIV-2 RT. The polyhistidine extension (His$_6$) at the N terminus of either HIV-1 or HIV-2 p51 RT allows rapid purification by metal chelate affinity chromatography on Ni$^{2+}$-NTA-Sepharose (16). By mixing a culture expressing this form of p51 with one expressing p66 lacking the extension, we predicted that both p51 and any reconstituted heterodimer would be retained by the matrix. Recently, this procedure has been employed to prepare selectively deuterated (p66/D-p51) HIV-1 RT for neutron solution scattering analysis. Enzyme thus prepared displays RNA-dependent DNA polymerase, RNase H, and tRNA$^{UUU}$ binding properties indistinguishable from our heterodimer RT generated by a twin RT-protease expression cassette (16). The presence of the small polyhistidine extension on one RT subunit therefore appears to affect neither the reconstitution process nor activity of the resulting enzyme. In the present communication, the reconstitution strategy has been extended to prepare heterodimeric HIV-2 RT as well as the chimeric heterodimers 1-p66-2-p51 and 2-p66-1-p51.

Reconstitution was also necessary for preparation of heterodimeric HIV-2 RT, since maturation of either the homologous or a chimeric HIV-1-HIV-2 pol gene in our E. coli expression system (23, 24) yielded only the 66-kDa subunit. In the p2RT-PROT expression cassette outlined in Fig. 1, the RT coding sequence was enzymatically excised from the HIV-2 pol gene such that it was preceded by 8 amino acids of protease and followed by 40 of integrase. As shown in Fig. 1B, co-expression of this ~72-kDa extended RT with HIV-1 protease results in elimination of the protease and integrase extensions, yielding the 66-kDa HIV-2 enzyme. Although we presently do not understand the inability of protease to release p51 HIV-2 RT, the p2RT-PROT twin expression cassette served as a convenient source of authentic p66 HIV-2 RT. The immunological analysis of Fig. 1B indicates that p66 HIV-2 RT can be detected with polyclonal antibodies against the p66 HIV-1 subunit, although the response is slightly reduced. Although appreciable expression of His$_6$-p51 HIV-2 RT was observed by Coomassie Blue staining of gels, its cross-reactivity with HIV-1 RT polyclonal antibodies was reduced relative to comparable amounts of the 66-kDa HIV-2 subunit, as well as either form of the HIV-1 enzyme.

Reconstitution of Homologous HIV-i and HIV-2 Heterodimers—Recovery of reconstituted heterodimeric p66-p51 HIV-1 RT from Ni$^{2+}$-NTA-Sepharose is summarized in Fig. 2. Since the reconstitution process is not quantitative, a portion of p66 is detected in the nonbinding fraction. However, due to its N-terminal polyhistidine extension, p51 RT is retained on the column and eluted only by application of an increasing gradient of imidazole. The same HIV-1 p51-containing fractions are also enriched for the poly-histidine-free HIV-1 66-kDa polypeptide, confirming that reconstitution between these RT subunits had occurred. In related experiments, reconstitution into heterodimer has been confirmed by interaction of RT with the HIV replication primer, tRNA$^{UUU}$ (13). Although the reassociation of p66 and p51 is qualitative, recovery of polyhistidine-extended p51 is quantitative, resulting in a slight over-representation of this subunit in fractions obtained by gradient elution.

Ni$^{2+}$-NTA-Sepharose chromatography of heterodimer HIV-2 RT reconstituted from combined lysates is presented in Fig. 3. Once again, free HIV-2 p66 is present in the nonbinding fraction, but a considerable portion is co-eluted with the polyhistidine-extended p51 subunit. In this experiment, it was interesting to observe that while two similarly sized "p66" polypeptides were present in the high speed supernatant, only the larger of these was selected by the 51-kDa HIV-2 polypeptide. This band is similar in size to p66 HIV-2 RT derived by proteolytic maturation from its pol gene precursor (23, 24), suggesting in the present analysis that only authentic p66 HIV-2 RT is selected by p51. In addition, this selective retention illustrates that the polyhistidine-free HIV-2 p66 is not retained on Ni$^{2+}$-NTA-Sepharose by nonspecific binding. The data of Figs. 2 and 3 illustrate that heterodimer reconstitution between homologous HIV-1 and HIV-2 RT subunits proceeds with little difficulty. The enzymatic activity associated with each heterodimer will be presented in a later section.

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J. L. Darlix, personal communication.

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\[ [A] \]

\[ \text{pRT} \] \[ \text{p6HRT51} \] \[ 1p66/1p51 \] \[ \text{p2RT-PROT} \] \[ \text{p6H2RT51} \] \[ 2p66/2p51 \] \[ \text{pRT} \] \[ \text{p6H2RT51} \] \[ 1p66/1p51 \] \[ \text{p2RT-PROT} \] \[ \text{p6HRT51} \] \[ 2p66/2p51 \]

\[ \text{B} \]

\[ \text{M} \] \[ 97 \] \[ 96 \] \[ 66 \] \[ 55 \] \[ 45 \] \[ 28 \] \[ 18 \] \[ \text{p66 HIV-1RT} \] \[ 1p51 HIV-1RT \] \[ 4 \] \[ 5 \] \[ \text{p51 HIV-2RT} \] \[ 1p51 HIV-2RT \]
Reconstitution of Chimeric Heterodimers—The apparent ease with which HIV-1 and HIV-2 RT subunits associate into homologous heterodimers prompted us to analyze whether the same approach might be effective for preparation of heterodimeric HIV RT comprised of a subunit from each enzyme. The protocol outlined in the previous section was applied to a combined homogenate containing HIV-1 p51 and HIV-2 p66 RT, the results of which are presented in Fig. 4A. 

As was observed during reconstitution of p66-p51 HIV-2 RT, a portion of the 66-kDa HIV-2 subunit, together with the slightly truncated form, was recovered in the nonbinding fraction. However, when protein eluted by imidazole gradient was analyzed, only a single fraction contained the reconstituted, chimeric heterodimer, and all other fractions were enriched for HIV-1 p51. Despite the low yield of reconstituted chimeric heterodimer, the results of Fig. 4A confirm that p66 HIV-2 RT alone has no affinity for Ni\textsuperscript{2+}-NTA-Sepharose. In a parallel experiment, the same pattern was observed using p51 HIV-1 RT and the p66 HIV-2 polypeptide released by maturation of the entire HIV-2 pol gene expressed from plasmid p2RTL1 (24) (data not shown). To determine whether reconstitution of nonhomologous RT subunits re-

**Fig. 2.** A, Ni\textsuperscript{2+}-NTA-Sepharose elution profile of a mixed pRT-p6HRT51 lysate (i.e., reconstitution of p66-p51 HIV-1 RT). Approximately 10 ml of mixed high speed supernatant was applied to a 4-ml column of Ni\textsuperscript{2+}-NTA-Sepharose at 5 ml/h. Following sample application, the column was washed with 10 ml of Buffer A/78, followed by ~12-column volumes of Buffer A/60 to remove loosely bound proteins. A gradient of 0-0.5 M imidazole in Buffer A/60 was then applied to elute the polyhistidine-extended p51 RT and any p66 with which it had reconstituted. B, immunological analysis of nonbinding (Buffer A/78, fractions 5-8) and imidazole-eluted samples (fractions 46-49). The number above each panel represents the column fraction tested. Lane M, prestained protein standards; lane C, control p66-p51 HIV-1 RT. The positions of the 66- and 51-kDa HIV-1 RT polypeptides in either the nonbinding or gradient-eluted fraction are indicated.

**Fig. 3.** Ni\textsuperscript{2+}-NTA-Sepharose purification of reconstituted HIV-2 p66-p51 RT. Only the immunological analysis has been presented. Upper, nonbinding fraction eluted with Buffer A/78. Lower, samples eluted with 0-0.5 M imidazole in Buffer A/60. For each analysis, the column fraction analyzed is indicated above the panel. M and C represent prestained protein markers and control p66-p51 HIV-1 RT, respectively. Migration positions of p66 and p51 HIV-2 RT have been indicated.

**Fig. 4.** A, immunological analysis of mixed HIV-2 p66-HIV-1 p51 RT lysate following Ni\textsuperscript{2+}-NTA-Sepharose chromatography. The buffer in which the samples were eluted from the column is indicated below the panel and the samples analyzed indicated above. Migration positions of p66 HIV-2 and p51 HIV-1 RT subunits are indicated. Protein M, markers are described in Fig. 3. B, rechromatography of mixed HIV-2 p66 and HIV-1 p51-containing samples on Ni\textsuperscript{2+}-NTA-Sepharose. Flow-through samples containing HIV-2 p66 and HIV-1 p51 were dialyzed, diluted with Buffer A/78, and allowed to incubate 12 h at 4 °C before being re-applied to Ni\textsuperscript{2+}-NTA-Sepharose. In this experiment, only the imidazole-eluted samples have been presented. Migration positions of the HIV-1 and HIV-2 RT subunits are indicated at the side of the panel.
quired prolonged preincubation prior to Ni\textsuperscript{2+}-NTA-Sepharose chromatography, we dialyzed the imidazole-eluted p51-containing samples into Buffer A/78, mixed the solution with samples of the nonbinding, 66 kDa-containing fraction (further purified by DEAE-Sepharose ion exchange chromatography), and allowed the reconstitution to proceed for 12 h at 4 °C prior to metal chelate affinity chromatography. Fig. 4b indicates that an early fraction of imidazole-eluted material was enriched for both p66 HIV-1 and p51 HIV-2 p66 RT and was followed by predominantly p51-containing samples. Based on this observation, we retained fraction 24 from the second round of chromatography as reconstituted chimeric heterodimer.

In Fig. 5, Ni\textsuperscript{2+}-NTA-Sepharose chromatography of combined lysates containing p66 HIV-1 and p51 HIV-2 RT is presented. As expected, a considerable proportion of HIV-1 p66 RT was recovered in the nonbinding fraction. In contrast to observations with its chimeric counterpart, HIV-2 p51 appears to associate more readily with the 66-kDa HIV-1 subunit. Although a second round of Ni\textsuperscript{2+}-NTA-Sepharose chromatography did not appear necessary from the data of Fig. 5, fractions 39-41 were pooled, dialyzed against Buffer A/78, and subjected to a second round of metal chelate affinity chromatography. Under these conditions, HIV-1 p66-HIV-2 p51 RT was again recovered from the imidazole-eluted fractions (data not shown), thereby eliminating the possibility of nonspecific binding of HIV-1 p66.

**Enzymatic Analysis of Homologous and Chimeric RT**—In a preliminary analysis, we determined whether the reconstituted enzymes regained RT activity. The activities reported in Table I indicate that although substantially different amounts of activity were recovered (between 3.74 and 84.9 units/µl), each RT preparation was nevertheless proficient in catalyzing DNA synthesis from a poly(rC)-oligo(dG) template-primed. When the specific activity of each preparation was determined, values of 1089 and 1522 units/mg were determined for the homologous HIV-1 and HIV-2 enzymes, respectively. Although we have achieved specific activities of 5000 units/mg for reconstituted, selectively deuterated HIV-1 heterodimer,\textsuperscript{1} the values reported here agree with our previous data using poly(rC)-oligo(dG) as template-primed (16). In contrast, the specific activity of each chimeric enzyme was almost 3-fold reduced (381 units/mg for 1-p66-2-p51 and 553 units/mg for 2-p66-1-p51) relative to its p66-containing counterpart. This result would suggest that, although chimeric enzyme reconstitution is possible, the orientation of the heterologous subunits may not be ideal.

Table II illustrates the extent to which each RT preparation could be inhibited by the TIBO derivative R82150. Pauwels et al. (17) have reported that this inhibitor is inactive towards HIV-2 RT but a potent inhibitor of the HIV-1 enzyme. This result is supported by the data of Table II, illustrating that R82150 concentrations up to 10 µM have little effect on HIV-2 p66-p51 but result in 90% inhibition of the heterodimeric HIV-1 enzyme.

Table II also illustrates the response of preparations of chimeric HIV-1-HIV-2 RT to inhibition by R82150. From these results, it is clear that this response is dependent upon the nature of the 66-kDa subunit, i.e. when this is from HIV-2, the enzyme is resistant to inhibition, and chimeric heterodimer bearing the HIV-1 66-kDa subunit is inhibited up to 90% by 10 µM R82150. The monophasic sensitivity of the chimeric enzymes to inhibition by R82150 strongly suggests that enzyme activity is not due to a mixture of active enzyme species, i.e. the contribution of homodimer p51 and p66 is negligible. Although several reports have indicated that recombiant p51 HIV RT is enzymatically inefficient (28-30), it is still possible that this subunit is active when a component of heterodimer. However, the results of Table II suggest that p51 is not likely to contribute significantly to the catalytic activity of heterodimer.

**DISCUSSION**

The rationale for this project was based on several independent observations regarding HIV-1 and HIV-2 RT. Pauwels et al. (17) have recently documented the TIBO derivative R82150, which displays a selective inhibition of HIV-1 replication in vitro. Similarly, Merluzzi et al. (18) and Wu et al. (19) have demonstrated selectivity of the inhibitor BI-RG-587 for HIV-1. These inhibitors act at the level of replication, and RT has been demonstrated as their target. At the same

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**Table I**

| Enzyme     | Activity (pmol/µl) | Specific activity (nmol/mg/20 min) | Ratio |
|------------|--------------------|-----------------------------------|-------|
| 1-p66-1-p51 | 84.9               | 1000                              | 1     |
| 1-p66-2-p51 | 3.74               | 381                               | 2.85  |
| 2-p66-2-p51 | 14.9               | 1522                              | 2.75  |
| 2-p66-1-p51 | 4.89               | 553                               |       |

*Specific activity ratio of homologous/chimeric p66-containing enzyme.

**Table II**

| Inhibition of HIV RT preparations by R82150 |
|---------------------------------------------|
| Relative activity                          |
| R82150                                     |
| 1-p66-1-p51 2-p66-2-p51 1-p66-2-p51 2-p66-1-p51 |
| µM  | 0.0  | 0.1  | 0.3  | 1.0  | 3.0  | 10.0 |
| %   | 100  | 100  | 100  | 104  | 90   | 104  |
|     | 71   | 90   | 100  | 93   | 89   | 89   |

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time, mutagenesis studies with recombinant HIV-2 p66 RT by Hizi et al. (31) indicated that RNase H activity of the HIV-2 enzyme was considerably lower than that of its HIV-1 counterpart. These independent observations illustrate that, despite considerable amino acid homology (~60%), there may be subtle structural differences between the HIV-1 and HIV-2 enzymes, necessitating the comparative study of each when developing a common inhibitor. In addition, Restle et al. (12) have demonstrated that dissociation of either heterodimeric (p66-p51) or homodimeric (p66-p66 and p51-p51) recombinant HIV-1 RT leads to loss of RNA-dependent DNA polymerase activity in the resulting monomer. From this latter study, it has been proposed that therapeutic agents might be designed that act to prevent dimerization of the 66- and 51-kDa HIV-1 and HIV-2 polypeptides. We therefore undertook to design an experimental strategy to address both dimerization of HIV RT and allow a comparative study of the HIV-1 and HIV-2 enzymes. This has been possible through reconstitution of homologous and chimeric heterodimeric enzymes.

Our data suggest that reconstitution of enzymatically active p66p51 HIV-1 and HIV-2 RT proceeds with relative ease. With respect to HIV-2 RT, this result has the practical advantage of permitting preparation of recombinant p66-p51 HIV-2 RT. In previous communications, we were unable to recover heterodimer HIV-2 RT via protease-mediated maturation of either its homologous (21) or a chimeric pol gene (22). In the present study, dissection of the HIV-2 pol gene to allow expression of its RT component together with HIV-1 protease has also failed to generate heterodimer. The reconstitution approach now makes preparation of the HIV-2 heterodimer possible through independent expression of its 66- and 51-kDa subunits, followed by their recovery from Ni²⁺-NTA-Sepharose. Although the present HIV-2 RT contains p51 in slight excess, S-Sepharose ion exchange chromatography has been used to separate reconstituted heterodimer HIV-1 RT from excess p51 and should be applicable to the HIV-2 enzyme. In contrast to their homologous p66-containing counterparts, chimeric enzymes display an approximately 3-fold reduction in specific activity, which may indicate that the dimer interfaces of heterodimer HIV-1 and HIV-2 RT are not identical.

Reconstituted HIV-1 and HIV-2 heterodimers exhibit the expected response to the TIBO derivative R82150, i.e., 10 μM R82150 has virtually no effect on HIV-2 RT but results in 90% inhibition of the RNA-dependent DNA polymerase activity of the HIV-1 enzyme. Furthermore, the response of chimeric heterodimers to R82150 strongly implicates the 66-kDa subunit as the target of this drug, i.e., heterodimers containing HIV-1 p66 (1-p66-1-p51 and 1-p66-2-p51) are sensitive to inhibition, whereas heterodimers containing a 66-kDa HIV-2 subunit (2-p66-2-p51 and 2-p66-1-p51) are resistant. The implication of heterodimer-associated p66 is in accordance with recent work indicating that either TTP or a synthetic template-primer is cross-linked selectively to p66 kDa of the HIV-1 heterodimer (32), as well as selective photoaffinity labeling of the p66 subunit of heterodimer with dipyriddiazepinone derivatives (19). In addition, recent cross-linking experiments involving the HIV replication primer tRNAs indicate that reduction in concentration of the cross-linking agent results in binding of tRNAs almost exclusively to heterodimer-associated p66.3 Taken together with analyses of purified recombinant p51 (28-30), these results suggest that heterodimer-associated p51 RT makes a minimal contribution to the activity of the enzyme. Unfortunately, neither the present results nor these recent observations go toward understanding the role of this RT subunit.

While p66 HIV-1 RT readily associates with the 51-kDa subunit of HIV-2, a surprising observation was the difficulty experienced in preparing the chimeric heterodimer 2-p66-1-p51. This may indicate subtle conformational differences between the HIV-1 and HIV-2 66-kDa subunits. In this respect, we have recently observed that a monoclonal antibody raised against HIV-1 p66 reacts with both HIV-1 subunits but selectively against HIV-2 p51. Since p51 HIV-2 RT is derived from the gene encoding p66, this observation supports the possibility that the 66-kDa subunits of HIV-1 and HIV-2 have subtly different conformations.

Finally, the ability to reconstitute heterodimeric HIV-1 and HIV-2 RT raises the possibility of establishing an assay system to monitor therapeutic agents that act by preventing dimer formation. Lysates containing homologous HIV-1 or HIV-2 RT subunits could be prepared in the presence of inhibitor, then subjected to Ni²⁺-NTA-Sepharose chromatography. Absence of the respective p66 subunit from the imidazole-eluted sample would be diagnostic of the inability to reconstitute heterodimer. With slight modifications, the process could also be adapted for batch analysis, permitting simultaneous analysis of several compounds. Since both HIV-1 and HIV-2 RT reconstitute rapidly, the effect of any particular agent on both HIV enzymes could be established.

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