Proc. Natl. Acad. Sci. USA
Vol. 88, pp. 9878–9882, November 1991
Medical Sciences

Chimeric human immunodeficiency virus type 1/type 2 reverse transcriptases display reversed sensitivity to nonnucleoside analog inhibitors

{AIDS/nevirapine (BI-RG-587)/tetrahydroimidazo[4,5,1-*jk*][1,4]benzodiazepin-2(1*H*)-one and -thione/3′-azido-2′,3′-dideoxythymidine}

CHENG-KON SHIH*, JANICE M. ROSE, GALE L. HANSEN, JOE C. WU, ALBINO BACOLLA, AND JOHANNA A. GRIFFIN

Boehringer Ingelheim Pharmaceuticals, Inc., 90 East Ridge, P.O. Box 368, Ridgefield, CT 06877

Communicated by Thomas A. Steitz, August 5, 1991 (received for review June 3, 1991)

ABSTRACT **Human immunodeficiency virus type 1 (HIV-1) reverse transcriptase (RT), an important therapeutic target in the treatment of AIDS, is effectively inhibited by a class of nonnucleoside analog compounds that includes nevirapine (BI-RG-587) and tetrahydroimidazo[4,5,1-*jk*]-[1,4]benzodiazepin-2(1*H*)-one and -thione. We show that both tyrosine residues at positions 181 and 188 flanking the putative catalytic site of HIV-1 RT are required for sensitivity of the enzyme to these compounds. HIV-2 RT, which does not have tyrosines at these positions, is resistant to these nonnucleoside analog inhibitors. Substitution of the HIV-2 RT amino acid residues at position 181 or 188 into HIV-1 RT results in an enzyme that is resistant to these compounds while retaining sensitivity to 3′-azido-2′,3′-dideoxythymidine triphosphate. HIV-2 RT substituted with amino acids 176–190 from HIV-1 RT acquires sensitivity to these nonnucleoside analog inhibitors.**

One of the early events in the establishment of infection by human immunodeficiency virus type 1 (HIV-1), the causative agent of AIDS, is the synthesis of double-stranded viral DNA that will become integrated into the genomic DNA of the host cell (1). This synthesis is catalyzed by the viral enzyme reverse transcriptase (RT), and inhibition of this enzymatic activity effectively blocks virus replication. Therefore, RT has become an important target for the development of drug therapy in the treatment of AIDS (1, 2). 3′-Azido-2′,3′-dideoxythymidine [AZT (zidovudine)], a nucleoside analog inhibitor of RT, was the first drug approved for AIDS therapy (2). It inhibits RT activity by competing with nucleotide binding to the enzyme, and it is incorporated into the growing DNA chain to prevent further chain elongation. This mechanism of inhibition is not specific for HIV-1 but broadly blocks other retroviruses as well. We recently described nevirapine, a potent, nonnucleoside analog inhibitor of HIV-1 RT-catalyzed synthesis of viral DNA; it inhibits enzyme activity *in vitro* and virus replication in tissue culture with 50% inhibitory concentrations (IC_{50} values) in the 40 nM range (3). This compound binds specifically to HIV-1 RT by a mechanism that is noncompetitive with respect to primer, template, nucleotide, and tRNA, and it does not inhibit HIV-2 RT or any other DNA polymerases tested (3, 4). A representativenonnucleosideanaloginhibitorinthetetrahydroimidazo[4,5,1-*jk*][1,4]benzodiazepin-2(1*H*)-one and -thione (TIBO) series of compounds (5) competed for the nevirapine binding site and probably inhibits by the same mechanism (4) (see Fig. 1 for structures of compounds). Therefore, it was not surprising that nevirapine displayed synergy with AZT and that AZT-resistant mutants of HIV-1 were sensitive to nevirapine in tissue culture experiments (6).

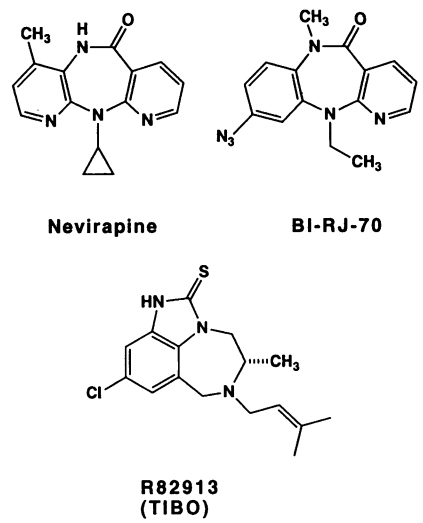

FIG. 1. Structures of nonnucleoside analog inhibitors of HIV-1 RT. Structures were published in the following references: nevirapine (BI-RG-587) and BI-RJ-70, refs. 3 and 4; TIBO, ref. 5.

The differential sensitivity of HIV-1 and HIV-2 to nevirapine provided us with a tool to better understand the mechanism by which these nonnucleoside analog inhibitors block RT-catalyzed DNA synthesis. We have generated chimeric enzymes by exchanging portions of the nevirapine binding domain from HIV-1 (7) and the corresponding domain from HIV-2 RT. Analyses of these chimeras have identified two specific amino acids from HIV-2 that, when substituted into HIV-1 RT, confer resistance to nevirapine and TIBO. They also defined a domain consisting of amino acids 176–190 from HIV-1 RT that, when substituted into HIV-2 RT, confers sensitivity to these two nonnucleoside analog inhibitors.

MATERIALS AND METHODS

Construction and Expression of Chimeric RTs. Schematic diagrams of the constructs are in Fig. 2. The HIV-1 RT expression clone, which is available from the National Institutes of Health AIDS Research and Reference Reagent Program, was obtained from Yale University (8). $HIV\text{-}2_{Rod}$

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: RT, reverse transcriptase; AZT (zidovudine), 3′-azido-2′,3′-dideoxythymidine; AZTTP, AZT triphosphate; PFA, phosphonoformic acid; HIV, human immunodeficiency virus; IPTG, isopropyl β-D-thiogalactopyranoside; TIBO, tetrahydroimidazo-[4,5,1-*jk*][1,4]benzodiazepin-2(1*H*)-one and -thione.

*To whom reprint requests should be addressed.

RT was expressed in the same vector as HIV-1 RT (J. Miglietta, personal communication). The construct, RT1 440, was made by overlap extension of primers using PCR (9, 10). All other mutant constructs were made by oligonucleotide-directed mutagenesis (11), using protocols available from commercial sources (Bio-Rad). Each RT construct was first cloned into the *Nco* I–*Hin*dIII sites of phagemid vector pGEM3Zf(+) (Promega) that was modified to contain an *Nco* I site in the polylinker region, and the clones were selected by nucleotide sequence analysis. Each chimeric construct was then subcloned into pKK233-2 (Pharmacia), the same expression vector as wild-type RTs. Mutations were again confirmed by nucleotide sequencing.

Lysates containing wild-type or mutant RTs were prepared by the following protocol: 5 ml of 2× YT medium (12) was inoculated with fresh overnight cultures of each clone and incubated at 37°C on a laboratory rotator (Glas-Col Apparatus, Terre Haute, IN). Isopropyl β-D-thiogalactopyranoside (IPTG) (Boehringer Mannheim) was added to a final concentration of 2.5 mM to induce the expression when cultures reached an OD_{600} of 0.6–0.9. After further incubation for 2 hr at 37°C cultures were centrifuged at 2700 × *g* for 10 min at 4°C to pellet bacterial cells. Pellets were resuspended in 500 μl of lysis buffer (0.5 M NaCl/10 mM Tris·HCl, pH 8/1 mM EDTA/0.5% Triton X-100/lysozyme, 2 mg/ml) and incubated on ice for 30 min. Samples were then sonicated for 30 sec (duty cycle, 50%; output, 20) on a Branson (Danbury, CT) Sonifier, transferred to Eppendorf tubes, and spun in a microcentrifuge at top speed for 10 min at 4°C. Supernatants were supplemented with glycerol to a final concentration of 10% and aliquoted for storage at −80°C until use.

Western Blot Analysis. Lysates from bacteria expressing RT constructs were run on an SDS/PAGE gel (13) and blot-transferred onto a nitrocellulose filter. The blot was allowed to react with an in-house-prepared monoclonal antibody directed against HIV-1 RT and developed with reagents and protocols from Promega. All constructs expressed predominantly p66 subunits in the bacterial lysates, with few detectable p51 subunits.

Enzyme Purification. Heterodimeric (p66/p51) RTs of wild-type HIV-1, HIV-2, and a mutant, RT1-Y181I, were purified to near homogeneity by a method described elsewhere (4). Small quantities of mutant RTs (RT1-Y181I, Y188L, and RT1 176–190) were purified from induced bacterial lysates by an immunoaffinity method essentially as described (14) and used for measuring IC_{50} for AZT triphosphate (AZTTP).

Binding of Photoaffinity Analog of Nevirapine. Sample RTs were labeled with [^{3}H]BI-RJ-70, a photoaffinity analog (see Fig. 1 for structure) that binds to the same site on HIV-1 RT as nevirapine under conditions in which the photoaffinity probe labeled only the p66, but not the p51, subunit of the wild-type HIV-1 RT heterodimer (4). Samples were then fractionated by SDS/PAGE, and the gel was fluorographed with Entensify (NEN) and exposed to x-ray film at −70°C for 3 days.

Polymerase and RNase H Assays. Nevirapine (3) and R82913, a TIBO derivative (5), were synthesized in-house. Phosphonoformic acid (PFA) was purchased from Fairfield Chemicals (Blythwood, SC). AZTTP was obtained from Raymond F. Schinazi through the AIDS Research and Reference Reagent Program, Division of AIDS, National Institute of Allergy and Infectious Diseases, National Institutes of Health. IC_{50} values for nevirapine, R82913, and PFA were derived from RNA-directed DNA polymerase assays, which were measured with bacterial lysates expressing wild-type or chimeric RTs and using poly(rC)·oligo(dG) as template primer as described (3). IC_{50} values for AZTTP were measured only with highly purified enzymes as indicated, using poly(rA)·oligo(dT) as template primer with the following reaction components: 50 mM Tris·HCl (pH 7.8), 60 mM NaCl, 2 mM $MgCl_2$, 5 nM poly(rA)$_{300}$·oligo(dT)$_{12-18}$ (Pharmacia), and 700 nM [^{3}H]TTP (NEN; specific activity, 20 Ci/mmol; 1 Ci = 37 GBq). IC_{50} measurements >200 μM were not pursued, because of the potential effects of high solvent concentration on the assay. Established protocols were used to assay DNA-directed DNA polymerase (3) and RNase H activities (14).

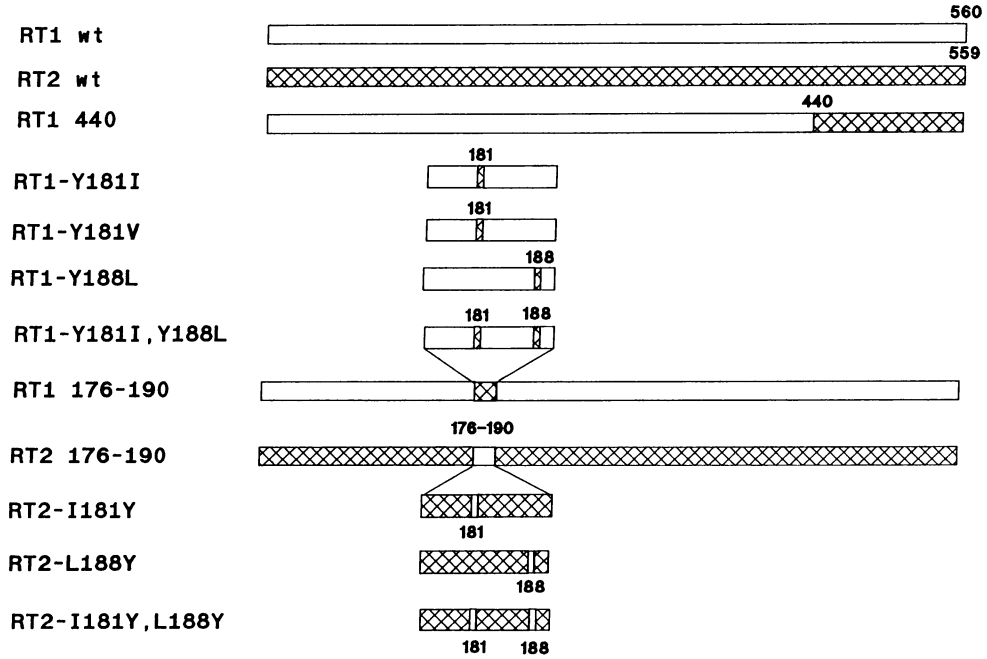

FIG. 2. Schematic representation of chimeric constructs between HIV-1 and HIV-2 RTs. Open boxes indicate the HIV-1 RT coding sequence. Shaded boxes indicate the coding sequences of HIV-2_{Rod} RT. Position numbers indicate amino acid residues at which the substitution was made—e.g., "RT1-Y181I" is HIV-1 RT in which tyrosine (Y) was replaced with isoleucine (I). Other abbreviations are V for valine and L for leucine. RT1 176–190 has amino acids 176–190, inclusive, from HIV-2 substituted into HIV-1 RT. The amino acid sequence of peptide 176–190 from HIV-1 RT is PDIVIYQYMDDLYVG and the sequence from HIV-2 RT is KDVIIIQYMDDILIA.

RESULTS

Chimeric Constructs in HIV-1 RT Background. Since nevirapine inhibits HIV-1 RT but not HIV-2 RT, enzymes that have 60% amino acid sequence identity, we made chimeric clones of HIV-1 and HIV-2 RT to analyze the mechanism by which these two enzymes differ in their sensitivity to nevirapine. We first constructed chimera RT1 440, in which the domain encoding RNase H activity in HIV-1 was exchanged for that domain of HIV-2 (Fig. 2). This clone had the nevirapine sensitivity pattern of wild-type HIV-1 RT (Table 1). This result suggested that the effect of the compound was in the polymerase, rather than in the RNase H, domain of the protein, so all further substitutions were restricted to the polymerase domain.

Photoaffinity mapping has localized the potential nevirapine binding sites to two tyrosine residues conserved in HIV-1 RT at positions 181 and 188 (7, 15). Although tyrosine residues are invariant at these positions in HIV-1 RT, isoleucine or valine at 181 and leucine at 188 are highly conserved in HIV-2 RT, which is resistant to nevirapine (15). We constructed single or double amino acid chimeras at these two positions in HIV-1 and HIV-2 RTs. Purified enzyme from the clone in which isoleucine was substituted for the tyrosine at position 181 in HIV-1 RT had RNA- and DNA-directed polymerase and RNase H activity similar to that of purified wild-type HIV-1 RT (data not shown). However, this chimeric RT was at least 200-fold less sensitive to nevirapine than wild-type HIV-1 RT (Table 1). DNA sequencing of the entire construct indicated that the only differences between the chimeric and parent clones were the expected TAT to ATT nucleotide substitutions. Back mutation of isoleucine to tyrosine fully restored HIV-1 RT sensitivity to nevirapine (Table 1). When we substituted valine into HIV-1 RT at position 181, we found that crude bacterial lysates containing this chimeric enzyme had similar polymerase activity as wild-type HIV-1 RT but had reduced nevirapine sensitivity similar to that of the isoleucine-substituted chimera. Finally, we substituted the leucine at position 188 from HIV-2 for the tyrosine at position 188 in wild-type and isoleucine chimeric HIV-1. The nevirapine sensitivity pattern of these chimeras was again like that of HIV-2 instead of HIV-1. Each substitution had a similar but less dramatic effect on TIBO sensitivity (Table 1). The presence of enzyme in each bacterial lysate was confirmed by Western blot analysis (Fig. 3). These results indicated that substitution of single amino acids from two different sites in HIV-2 RT into the HIV-1 RT altered sensitivity of the enzyme to two structurally distinct nonnucleoside analog RT inhibitors. Interestingly, enzymes from the chimeric constructs tested against PFA retained their sensitivity, and chimeric HIV-1 RTs were as sensitive as the wild-type RT to AZTTP (Table 1).

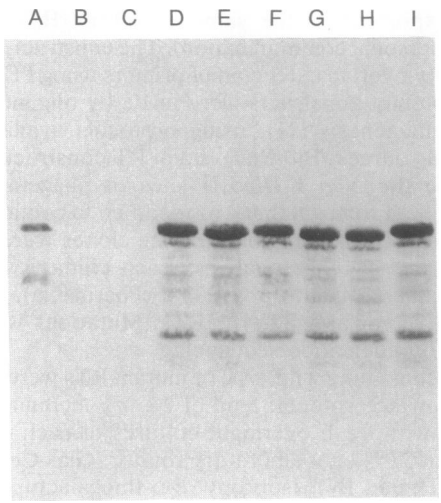

FIG. 3. Western blot analysis of HIV-1/HIV-2 RT chimeras. Purified HIV-1 RT heterodimer (lane A) indicates the position for p66 and p51. Purified HIV-2 RT (lane B) and crude bacterial lysate containing the expression vector alone (lane C) do not react with the monoclonal antibody. Lanes C–I are crude bacterial lysates containing the indicated constructs: pKK233-2 vector (Pharmacia) alone (lane C); wild-type HIV-1 RT (lane D); Y181I (lane E); Y181V (lane F); Y188L (lane G); Y181I, Y188L (lane H); I181Y back mutation (lane I). Lanes E–I are all constructed in the HIV-1 RT background.

Binding of Photoaffinity Analog of Nevirapine to Mutant RTs. To determine whether the loss of sensitivity to nevirapine was due to the inability of the compound to bind to the position 181 isoleucine and valine and the position 188 leucine chimeras, the chimeras were allowed to react with BI-RJ-70, a photoaffinity analog that competes with the binding of nevirapine to wild-type HIV-1 RT with an IC_{50} of 0.085 ± 0.01 μM (4). The [^{3}H]BI-RJ-70 reacted strongly with the purified wild-type HIV-1 enzyme as well as with the wild-type HIV-1 enzyme in crude bacterial lysates (Fig. 4, lanes A and D). There was no reactivity between BI-RJ-70 and wild-type HIV-2 or any of the chimeras modified at amino acid 181 (Fig. 4, lanes B, E, F, and H). The mutation of the isoleucine back to the wild-type tyrosine at position 181 restored strong reactivity with the labeled photoaffinity analog (Fig. 4, lane I), indicating that it was the single amino acid substitution that prevented the chimera from reacting. These findings suggested that the resistance of the position 181 chimeric RTs and HIV-2 RT to nevirapine was due to the inability of the

Table 1. IC_{50} values for HIV-1/HIV-2 RT chimeras

Enzyme*	Nevirapine, μM	R82913, μM	PFA, μM	AZTTP, μM
RT1 wild-type	0.25 ± 0.01	1.7 ± 0.1	29 ± 6	0.05 ± 0.01
RT1 440	0.11 ± 0.01	ND	24 ± 12	ND
RT1-Y181I	73 ± 4	36 ± 1	65 ± 30	0.02 ± 0.01
RT1-I → Y†	0.28 ± 0.05	ND	20 ± 3	ND
RT1-Y181V	56 ± 4	60 ± 10	17 ± 10	ND
RT1-Y188L	>200	60 ± 10	17 ± 10	ND
RT1-Y181I, Y188L	>200	>200	60 ± 8	0.02 ± 0.01
RT1 176–190	>200	>200	ND	0.02 ± 0.01
RT2 wild-type	>200	>200	4.8 ± 0.7	0.11 ± 0.03
RT2-I181Y	>200	>200	ND	ND
RT2-L188Y	>200	>200	ND	ND
RT2-I181Y, L188Y	121 ± 21	8.2 ± 0.4	ND	ND
RT2 176–190	0.8 ± 0.1	0.52 ± 0.10	ND	ND

ND, not determined.
*See Fig. 2 for mutant construction.
†The symbol I → Y indicates the back mutation of mutant I-181 to wild-type residue.

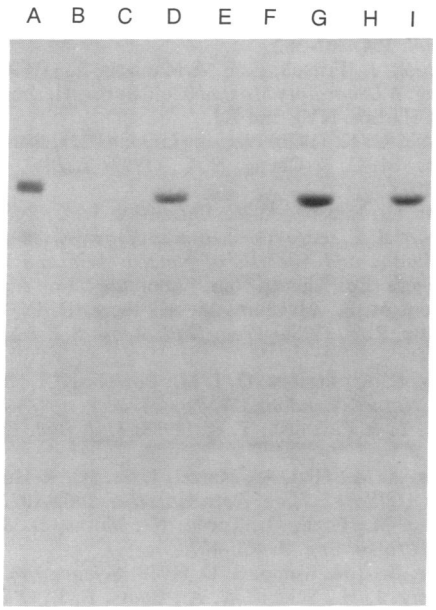

FIG. 4. Binding of photoaffinity analog of nevirapine. RT samples labeled with [^{3}H]BI-RJ-70 were run on a SDS/PAGE gel and autoradiographed. Lanes A–I are the same as those described in the legend to Fig. 3. Each sample was run at least twice.

enzymes to interact with the compound. Surprisingly, the photoaffinity probe reproducibly reacted with the chimera containing the leucine substitution at position 188 (Fig. 4, lane G), even though that chimeric enzyme had dramatically reduced sensitivity to BI-RJ-70 (IC_{50} = 7.4 ± 1.4 μM) and had lost all detectable sensitivity to nevirapine. Therefore, binding of BI-RJ-70 to this mutant HIV-1 RT was not sufficient for the compound to inhibit activity of the enzyme.

Chimeric Constructs in HIV-2 RT Background. We performed reciprocal exchanges in HIV-2 RT to see if the HIV-1-derived amino acids could confer sensitivity to nevirapine and TIBO. The substitution of tyrosine into HIV-2 RT at residue 181 or 188 resulted in chimeras in crude bacterial lysates that had comparable activity to wild-type HIV-2 RT in the RNA-directed DNA polymerase assay. These chimeras, like wild-type HIV-2 RT, were insensitive to nevirapine and TIBO (Table 1). The HIV-2 RT enzyme with position 181 and 188 double mutations reproducibly gained a small increase in sensitivity to nevirapine and a rather significant increase in sensitivity to TIBO. These data indicated that the tyrosines at positions 181 and 188 were required for inhibition of HIV-1 RT by nevirapine, but the presence of those residues in HIV-2 RT was not sufficient to mediate full sensitivity to the compound.

Since substitutions at amino acids 181 and 188 alone were adequate to abolish sensitivity of HIV-1 RT but not to render HIV-2 RT completely sensitive to nevirapine and TIBO, we exchanged the entire segment of amino acids 176–190 between HIV-1 and HIV-2 (Fig. 2). The resulting chimeric enzymes had a nonnucleoside analog sensitivity pattern similar to that of the donor of the 176–190 peptide—i.e., HIV-1 RT containing the HIV-2 segment was not sensitive to either compound, and HIV-2 RT containing the HIV-1 insert was fully sensitive to both compounds (Table 1). These results indicated that the effective interaction of nevirapine and TIBO with RT required not only the two tyrosines at positions 181 and 188 but also the microstructure around them.

DISCUSSION

Tyrosines 181 and 188 of HIV-1 RT Are Necessary for Sensitivity to Nonnucleoside Analog Inhibitors. There is strict conservation of tyrosine at positions 181 and 188 that flank the putative active site of HIV-1 RT (16–20). In contrast, the amino acids isoleucine/valine and leucine at positions 181 and 188, respectively, are highly conserved in HIV-2 RT. Our data demonstrated that if these amino acids replace the tyrosines in HIV-1 RT, it too became resistant to the nonnucleoside analog inhibitors nevirapine and TIBO. These results suggested that the presence of tyrosine residues at positions 181 and 188 in HIV-1 RT is necessary for sensitivity to these nonnucleoside analog inhibitors. The results also suggested that alteration at these binding sites might be one of the mechanisms by which resistant variants of HIV-1 can arise. Since two nucleotide changes in the tyrosine codon were required to produce amino acid substitutions of isoleucine, valine, or leucine, we speculate that HIV-1 variants with these specific mutations in RT should occur relatively infrequently.

The observation that the photoaffinity analog BI-RJ-70 could bind to mutant HIV-1 RT containing the tyrosine to leucine substitution at amino acid 188, but was 100-fold less effective in inhibiting the polymerase activity of that mutant, is something of a paradox. One possible explanation for this curious result is that there is a preferred orientation of the compound in its interaction with the protein such that the asymmetrically located azido probe (Fig. 1) is most often in close proximity to tyrosine 181. Other possibilities may include the differential accessibility of tyrosine 181 compared with that of tyrosine 188 or a mutation-induced change in the conformation in the binding domain.

Peptide 176–190 of HIV-1 RT Renders HIV-2 RT Sensitive to Nevirapine and TIBO. Our data demonstrated that the region 176–190 of HIV-1 RT confers sensitivity of HIV-2 RT to nevirapine and TIBO. On the other hand, the reciprocal enzyme construct, RT1 176–190, which had 176–190 region from HIV-2 RT, remained as sensitive to AZTTP as wild-type HIV-1 RT, despite its loss of all sensitivity to nevirapine and TIBO. These results were consistent with and further extended the observation that nevirapine and TIBO, although structurally distinct compounds, bind to the same domain in HIV-1 RT, as described by competition studies (4). Our results were also consistent with the observations that nevirapine was effective against AZT-resistant strains of HIV-1 in tissue culture (6) and that amino acid residues conferring AZT resistance mapped to sites outside of amino acids 176–190 (21). Our results further implied that HIV-1 variants resistant to the nonnucleoside analog inhibitors nevirapine and TIBO would not acquire cross-resistance to AZT or even other nucleoside analog inhibitors.

These chimeric enzymes and the insights they provide for understanding the mechanism of inhibition of HIV-1 RT by nevirapine will be powerful tools in identifying second generation nonnucleoside analog inhibitors of HIV-1 and HIV-2 RTs. In addition, these studies have identified specific sites to be monitored for the diagnosis/characterization of clinical isolates from AIDS patients that might become resistant to nonnucleoside analog therapy.

Note Added in Proof. Our hypothesis that HIV-1 variants resistant to nevirapine may arise by alteration at the binding sites of RT was confirmed by several independent studies in cell culture (refs. 22 and 23; Yung-chi Cheng, personal communication), in which a single nucleotide change of A to G at RT codon 181 (from tyrosine TAT to cysteine TGT) rendered the virus resistant to nevirapine and other nonnucleoside RT inhibitors.

We thank J. Miglietta, A. Shrutkowski, S. Goldrick, K. Lubbe, G. Caviness, J. Glinski, T. Warren, S. Rogers, M. Czajkowski, R. Rothlein, and P. Grob for reagents; J. Sabo for analysis of results; K. Hargrave, J. Proudfoot, and J. Klunder for nevirapine, BI-RJ-70, TIBO, and their structures; and W. Hayward, J. Adams, P. Farina, and A. Rosenthal for helpful discussions.

1. Goff, S. P. (1990) *J. Acquired Immune Defic. Syndr.* **3,** 817–831.
2. Mitsuya, H., Yarchoan, R. & Broder, S. (1990) *Science* **249,** 1533–1544.
3. Merluzzi, V. J., Hargrave, K. D., Labadia, M., Grozinger, K., Skoog, M., Wu, J. C., Shih, C.-K., Eckner, K., Hattox, S., Adams, J., Rosenthal, A. S., Faanes, R., Eckner, R. J., Koup, R. A. & Sullivan, J. L. (1990) *Science* **250,** 1411–1413.
4. Wu, J. C., Warren, T. C., Adams, J., Proudfoot, J., Skiles, J., Raghavan, P., Perry, C., Potocki, I., Farina, P. R. & Grob, P. M. (1991) *Biochemistry* **30,** 2022–2026.
5. Pauwels, R., Andries, K., Desmyter, J., Schols, D., Kukla, M. J., Breslin, H. J., Raeymaeckers, A., Gelder, J. V., Woestenborghs, R., Heykants, J., Schellekens, K., Janssen, M. A. C., De Clercq, E. & Janssen, P. A. J. (1990) *Nature (London)* **343,** 470–474.
6. Richman, D., Rosenthal, A. S., Skoog, M., Eckner, R. J., Chou, T.-C., Sabo, J. P. & Merluzzi, V. J. (1991) *Antimicrob. Agents Chemother.* **35,** 305–308.
7. Cohen, K. A., Hopkins, J., Ingraham, R. H., Pargellis, C., Wu, J. C., Palladino, D. E. H., Kinkade, P., Warren, T. C., Rogers, S., Adams, J., Farina, P. R. & Grob, P. M. (1991) *J. Biol. Chem.* **266,** 14670–14674.
8. D'Aquila, R. T. & Summers, W. C. (1989) *J. Acquired Immune Defic. Syndr.* **2,** 579–587.
9. Ho, S. N., Hunt, H. D., Horton, R. M., Pullen, J. K. & Pease, L. R. (1989) *Gene* **77,** 51–59.
10. Horton, R. M., Hunt, H. D., Ho, S. N., Pullen, J. K. & Pease, L. R. (1989) *Gene* **77,** 61–68.
11. Kunkel, T. A., Roberts, J. D. & Zakour, R. A. (1987) *Methods Enzymol.* **154,** 367–382.
12. Sambrook, J., Fritsch, E. F. & Maniatis, T. (1989) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Lab., Cold Spring Harbor, NY), 2nd Ed.
13. Laemmli, U. K. (1970) *Nature (London)* **227,** 680–685.
14. Starnes, M. C. & Cheng, Y.-C. (1989) *J. Biol. Chem.* **264,** 7073–7077.
15. Meyers, G., Rabson, A. B., Berzofsky, J. A., Smith, T. F. & Wong-Staal, F., eds. (1990) *Human Retroviruses and AIDS, a Compilation and Analysis of Nucleic Acid and Amino Acid Sequences* (Los Alamos Natl. Laboratory, Los Alamos, NM).
16. Johnson, M. S., McClure, M. A., Feng, D.-F., Gray, J. & Doolittle, R. F. (1986) *Proc. Natl. Acad. Sci. USA* **83,** 7648–7652.
17. Larder, B. A., Purifoy, D. J. M., Powell, K. L. & Darby, G. (1987) *Nature (London)* **327,** 716–717.
18. Xiong, Y. & Eickbush, T. H. (1988) *Mol. Biol. Evol.* **5,** 675–690.
19. Barber, A. M., Hizi, A., Maizel, J. V., Jr., & Hughes, S. H. (1990) *AIDS Res. Hum. Retroviruses* **6,** 1061–1072.
20. Delarue, M., Poch, O., Tordo, N., Moras, D. & Argos, P. (1990) *Protein Eng.* **3,** 461–467.
21. Larder, B. A. & Kemp, S. D. (1989) *Science* **246,** 1155–1158.
22. Nunberg, J. H., Schleif, W. A., Boots, E. J., O'Brien, J. A., Quintero, J. C., Hoffman, J. M., Emini, E. A. & Goldman, M. E. (1991) *J. Virol.* **65,** 4887–4892.
23. Richman, D., Shih, C.-K., Lowy, I., Rose, J., Prodanovich, P., Goff, S. & Griffin, J. (1991) *Proc. Natl. Acad. Sci. USA*, in press.