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Viral Resistance to Human Immunodeficiency Virus Type 1-Specific Pyridinone Reverse Transcriptase Inhibitors

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Human immunodeficiency virus type 1 (HIV-1)-specific pyridinone reverse transcriptase (RT) inhibitors prevent HIV-1 replication in cell culture (M. E. Goldman, J. H. Nunberg, J. A. O'Brien, J. C. Quintero, W. A. Schleif, K. F. Freund, S. L. Gaul, W. S. Saari, J. S. Wai, J. M. Hoffman, P. S. Anderson, D. J. Hupe, E. A. Emini, and A. M. Stern, Proc. Natl. Acad. Sci. USA 88:6863–6867, 1991). In contrast to nucleoside analog inhibitors, such as AZT, which need to be converted to triphosphates by host cells, these compounds act directly to inhibit RT via a mechanism which is noncompetitive with respect to deoxynucleoside triphosphates. As one approach to define the mechanism of action of pyridinone inhibitors, we isolated resistant mutants of HIV-1 in cell culture. Serial passage in the presence of inhibitor yielded virus which was 1,000-fold resistant to compounds of this class. Bacterially expressed RTs molecularly cloned from resistant viruses were also resistant. The resistant RT genes encoded two amino acid changes, K-103 to N and Y-181 to C, each of which contributed partial resistance. The mutation at amino acid 181 lies adjacent to the conserved YG/MDD motif found in most DNA and RNA polymerases. The mutation at amino acid 103 lies within a region of RT which may be involved in PP_i binding. The resistant viruses, although sensitive to nucleoside analogs, were cross-resistant to the structurally unrelated RT inhibitors TIBO R82150 (R. Pauwels, K. Andries, J. Desmyter, D. Schols, M. J. Kukla, H. J. Breslin, A. Raeymaeckers, J. Van Gelder, R. Woestenborghs, J. Heykanti, K. Schellekens, M. A. C. Janssen, E. De Clercq, and P. A. J. Janssen, Nature [London] 343:470–474, 1990) and BI-RG-587 (V. J. Merluzzi, K. D. Hargrave, M. Labadia, K. Grozinger, M. Skoog, J. C. Wu, C.-K. Shih, K. Eckner, S. Hattox, J. Adams, A. S. Rosenthal, R. Faanes, R. J. Eckner, R. A. Koup, and J. L. Sullivan, Science 250:1411–1413, 1990). Thus, these nonnucleoside analog inhibitors may share a common binding site on RT and may all make up a single pharmacologic class of RT inhibitor. This observation may have important implications for the clinical development of these compounds.

Human immunodeficiency virus type 1 (HIV-1) is the major etiologic agent of the acquired immunodeficiency syndrome (AIDS) (4, 10). Infection results in progressive destruction of $CD4^+$ T lymphocytes and inexorable collapse of immune function (8). Antiviral therapies using nucleoside analogs directed against the viral reverse transcriptase (RT) have shown promise in delaying the progression of disease (5, 9). We and colleagues have recently described a new class of RT inhibitors—pyridinone-containing compounds which specifically inhibit HIV-1 RT (11). The more potent members of this class are active at concentrations in the nanomolar range to inhibit the spread of HIV-1 infection in cell culture. An antiviral effect is observed in human T-lymphoid cell lines and in human peripheral blood T lymphocytes, and it extends to geographically diverse strains of HIV-1 and to primary isolates from patients. The mechanism of RT inhibition by these compounds is unknown. These compounds do not act as nucleoside analogs but rather bind directly to the functional RT complex (RT-template–primer-nucleotide) and act to inhibit polymerization in a manner which is noncompetitive with respect to either the nucleotide or the template-primer (11). As one approach to explore the mechanism of action of these novel inhibitors and to define specific regions of RT which mediate this inhibition, we isolated in cell culture and characterized an HIV-1 strain that was resistant to the antiviral effect of these compounds.

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MATERIALS AND METHODS

Pyridinone RT inhibitors. The class of HIV-1-specific pyridinone RT inhibitors has already been described (11). The specific compounds used in our studies are presented in Fig. 1.

Cell culture growth and antiviral testing of HIV-1. Resistant variants of HIV-1 strain IIIb were isolated in H9 human T-lymphoid cell culture (26). Selection involved initial passage of the virus in medium containing 40 μM L-693,593, followed by three serial passages in medium containing 100 μM L-693,593. For each passage, cultures were infected by using a 1:2, 1:5, or 1:25 dilution of supernatant from the preceding passage (2,000 to 300 ng of HIV-1 p24 per ml of culture). The virus inoculum was removed after 24 h, and cultures were continued in the presence of inhibitor. The culture which showed an initial multiplicity of infection of ≤1% in the presence of inhibitor and attained 100% infection in approximately 14 days was chosen for subsequent passage. In this manner, we sought to balance the need for multiple rounds of replication with the need for genetic diversity in each inoculum. Spread of infection was determined by indirect immunofluorescence using serum from an HIV-1-infected individual. HIV-1 p24 core antigen was determined by enzyme-linked immunosorbent assay (Coulter Immunology).

MT-4 T-lymphoid cells (23) were used to determine the sensitivity of HIV-1 to RT inhibitors. Cells were infected by using a predetermined inoculum, and cultures were incubated for 24 h. At this time, ≤1% of the cells were positive

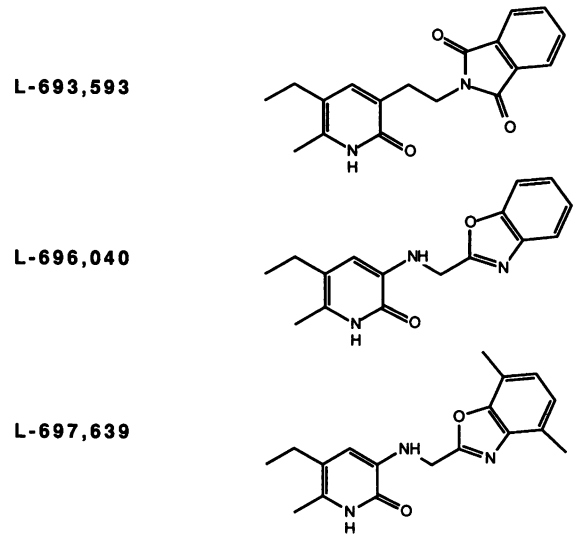

FIG. 1. HIV-1-specific pyridinone RT inhibitors. Compounds: L-693,593, 5-ethyl-6-methyl-3-(2-phthalimidoethyl)pyridin-2(1H) one; L-696,040, 3-{[(1,3-benzoxazol-2-yl)methyl]amino}-5-ethyl-6-methyl-pyridin-2(1H)one; and L-697,639, 3-{[(4,7-dimethyl-1,3-benzoxazol-2-yl)methyl]amino}-5-ethyl-6-methyl-pyridin-2(1H)one.

by indirect immunofluorescence. Cells were then extensively washed and distributed into 96-well culture dishes. Serial twofold dilutions of inhibitor were added to the wells, and cultures were continued for 3 additional days. At 4 days postinfection, 100% of the cells in control cultures were infected. HIV-1 p24 accumulation was directly correlated with virus spread. The cell culture inhibitory concentration was defined as the inhibitor concentration which reduced the spread of infection by at least 95%. Because all virus populations appeared monodisperse and displayed parallel dose-response curves, we were able to quantify resistance as the ratio of inhibitor concentrations that reduced the spread of infection by at least 95%. Recombinant HIV-1 was generated by calcium phosphate-mediated transfection (13) of proviral DNA into CD4-expressing HeLa HT4-6C cells (6). The HT4-6C cell line was obtained from Bruce Chesebro through the National Technical Information Service. Progeny virus was amplified by cocultivation by using FDA-H9 cells kindly provided by Barbara J. Potts (Repligen Corp.). Molecularly cloned infectious provirus NL4-3 (1) was obtained from Malcolm Martin (National Institutes of Health).

Nucleic acid techniques. RT genes from resistant viruses were molecularly cloned by using the polymerase chain reaction (PCR) and thermostable *Taq* polymerase (Cetus-Perkin Elmer) (7, 31). trpE-RT expression vector pHRTRX2 (36) was obtained through the AIDS Research and Reagent Program, AIDS Program, National Institute of Allergy and Infectious Diseases, from Stephen Goff (Columbia University). Synthetic oligonucleotide primers (Synthecell Corp., Rockville, Md.) were designed to facilitate subsequent molecular cloning in pHRTRX2. The 5′ primer 5′-cgcggatccgc gACTTTAAATTTTCCCATTAG encoded 4 amino acids of HIV-1 protease (in contrast to the 13 amino acids encoded in pHRTRX2 [27]) and 2 2/3 amino acids of HXB2 RT and contained a 5′ extension (lowercase letters) to facilitate molecular cloning at the *Bam*HI site of pHRTRX2. The 3′ primer 5′-ctagtctagactagGGGCCTTATCTATTCCATC encoded six and one-third amino acids of integrase and contained a 5′ extension (lowercase letters) to allow molecular cloning at the *Xba*I site of pHRTRX2. Because the 5′ PCR primer differed from that of pHRTRX2, we were able to confirm the replacement of the original HXB2 RT gene with the PCR product by *Hin*fI restriction endonuclease analysis. Molecular cloning and other nucleic acid techniques were done as previously described (21). Nucleic acid enzymes were from New England BioLabs, Inc., Beverly, Mass., or Bethesda Research Laboratories, Inc., Gaithersburg, Md. BlueScript plasmid vectors (Stratagene, La Jolla, Calif.) were used for molecular cloning of PCR products. DNA sequence analysis (32) used Sequenase T7 DNA polymerase (U.S. Biochemical Corp., Cleveland, Ohio).

Analysis of bacterially expressed RT. *Escherichia coli* HB101 containing pHRTRX2-derived expression plasmids was grown in M9 minimal medium with supplements, including 50 μg of tryptophan per ml. Expression was induced by tryptophan starvation and addition of indoleacrylic acid as previously described (37). Cells were treated with lysozyme (20 μg/ml) prior to lysis using 0.1% Triton X-100 and mild sonication. Soluble extracts were obtained by centrifugation.

RT assays were carried out in 55 mM Tris (pH 8.2)–30 mM KCl–30 mM $MgCl_2$–1 mM dithiothreitol–20 μg of rC:dG_{12-18} (Pharmacia) per ml–8 μM [^{3}H]dGTP (New England Nuclear)–0.01% Triton X-100–50 μM ethylene glycol-bis(β-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid (EGTA)–1 mg of bovine serum albumin per ml. After 60 min of incubation at 37°C, acid-precipitable material was collected onto glass fiber filters by using a Skatron semiautomatic cell harvester (11). Bacterial cell extracts containing RT were diluted to within the linear range of the assay, and activity was determined in the presence and absence of inhibitor. Purified HIV-1 RT heterodimer produced in *E. coli* served as the standard (12).

Nucleotide sequence accession numbers. The nucleotide sequences of the resistant A17 and B17 RT genes have been submitted to GenBank under accession numbers M69212 and M69213, respectively.

RESULTS

Isolation of resistant viruses. L-693,593 is a member of the pyridinone class of HIV-1-specific RT inhibitors (Fig. 1). This compound at 40 μM inhibits the spread of HIV-1 infection in H9 human T-lymphoid cell cultures by ≥95% (11). Selection for resistance to L-693,593 entailed serial passage of HIV-1 strain IIIb in H9 cells in the presence of increasing concentrations of the compound. Selection was initiated in medium containing 40 μM L-693,593 and continued through three serial passages in medium containing 100 μM L-693,593. The first difference in the growth of the selected population relative to the original HIV-1 population was observed at passage 3. Figure 2 demonstrates the growth advantage of the selected population at passage 4 in 100 μM L-693,593.

Infected cells from passage 4 were cloned in the presence of 100 μM L-693,593 into 96-well culture plates containing uninfected FDA-H9 cells. The FDA-H9 T-lymphoid cell line is an early clone of H9 obtained by the Bureau of Biologicals, U.S. Food and Drug Administration, and was kindly provided by Barbara J. Potts. This cell line demonstrates extensive syncytium formation upon HIV-1 infection, and thus, wells containing infected cells could be determined by microscopic examination. In medium containing 100 μM L-693,593, the efficiency of infection by cells from the

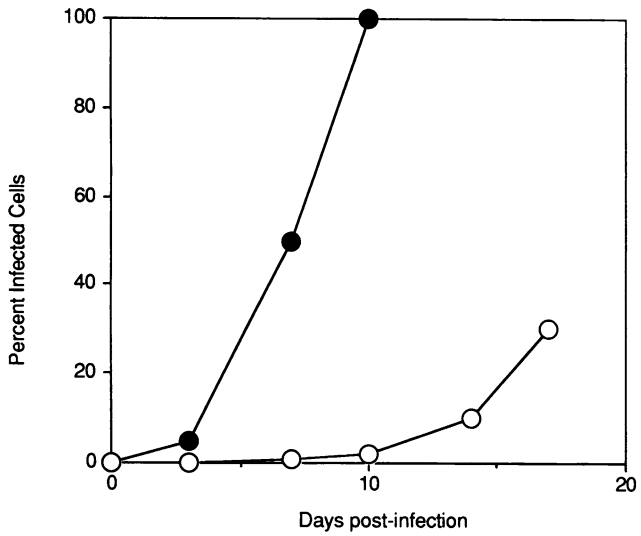

FIG. 2. Growth of passage 4 virus in medium containing 100 μM L-693,593. H9 cells were infected by using cell culture supernatants from the passage 4 culture (●) or from H9 cells infected with the parental HIV-1 population (○). Each inoculum contained approximately 150 ng of HIV-1 p24. Spread of infection was monitored by indirect immunofluorescence.

passage 4 culture was ~85% (41 of 120 wells versus 24 of 60 wells in a parallel cloning performed in the absence of inhibitor). Cells infected with the original HIV-1 population established only a limited infection, with ≤15% efficiency. Infected FDA-H9 cell cultures derived from cloned passage 4 cells were expanded to establish persistently infected cell lines. This cloning procedure is rapid and approximates the more rigorous biological cloning of virus by repeated end-point titration.

Characterization of resistant viruses and RTs. Resistant viruses from two persistently infected cell lines, A17 and B17, were chosen for further analysis in MT-4 human T-lymphoid cell culture. Table 1 summarizes the specific resistance of clone A17 virus to two additional members of the pyridinone class of RT inhibitors, L-696,040 and L-697,639. A17 was approximately 1,000-fold less sensitive to these inhibitors than was the parental HIV-1. Despite high levels of resistance to the pyridinone inhibitors, A17 showed no change in sensitivity to two nucleoside analog RT inhibitors, zidovudine (AZT) and dideoxyinosine. Comparable results were obtained with B17 virus. The growth properties of virus obtained from the A17 and B17 cell lines were indistinguishable from those of the parental HIV-1.

The RT genes from the L-693,593-resistant proviruses were molecularly cloned by PCR. To facilitate subsequent expression of the RT proteins in *E. coli*, primers were designed on the basis of the *trpE*-RT gene fusion construction in plasmid pHRTRX2 (36). The specific 1.8-kb PCR product was purified by gel electrophoresis and molecularly cloned by using a BlueScript SK plasmid. Colonies harboring plasmids with inserts were pooled (20 to 40 per pool), and the population was used to prepare the adapted RT gene for insertion into the expression vector.

TABLE 1. Sensitivities of A17 and HIV-1 IIIb viruses

Compound	CIC_{95}[a] (nM)		Fold resistance
	A17	HIV-1 IIIb	
L-696,040	100,000	200	500
L-697,639	25,000	25	1,000
AZT	25	25	1
Dideoxyinosine	6,250	6,250	1

[a] CIC_{95}, inhibitor concentration that reduced the spread of infection by at least 95%.

Five clones from each experiment were chosen, and the sensitivity of the bacterially expressed RT to L-693,593 was analyzed. Most of the molecular clones yielded RT activity. These all produced enzyme that was resistant to 100 μM L-693,593 (≤10% inhibition). The RT derived from pHRTRX2 or from the PCR product of parental HIV-1 was sensitive to the inhibitor (≥90% inhibition). A17 and B17 RTs were unchanged in their sensitivities to the PP_i analog phosphonoformate and to the triphosphates of the nucleoside analogs AZT, dideoxyguanosine, and dideoxythymidine. The concordance observed between the resistance of the virus and that of the associated RT enzyme is in marked contrast to descriptions of AZT-resistant viruses in which the RT enzyme remains sensitive to AZT triphosphate (17, 29).

To define the genetic changes responsible for resistance, the DNA sequences of the resistant RT genes were determined (Fig. 3). In both A17 and B17, single-base-pair changes resulted in conversion of K-103 to N (AAA to AAC) and Y-181 to C (TAT to TGT) within the polymerase domain of the protein (3, 35). Other nucleotide changes were silent, except for two encoding changes in the RNase H domain of RT (15) (R-461 to K and Q-512 to K) and one encoding a change unique to B17 (H-221 to Y).

Restriction endonuclease fragments encoding one or more of the amino acid changes were exchanged into the wild-type HXB2 RT of pHRTRX2 to determine the changes responsible for resistance (Fig. 4). The intact RT of A17 was approximately 2,000-fold resistant to the potent pyridinone inhibitor L-697,639. Subdivision at a unique *Pfl*MI restriction endonuclease site localized the full extent of resistance to the amino-terminal portion of the molecule containing the K-103-to-N and Y-181-to-C changes (*Bam*HI to *Pfl*MI; amino acids 1 to 315). Reciprocal exchanges using the *Eco*RV restriction endonuclease site (amino acid 144) revealed that RTs containing the K-103-to-N mutation were 20-fold resistant, while RTs containing the Y-181-to-C mutation were 800-fold resistant. Thus, both the K-103-to-N and Y-181-to-C mutations contributed partial resistance; the combination of the two mutations was more than additive, resulting in ~2,000-fold resistance. The other amino acid changes did not appear to affect resistance. The changes involved in resistance to L-693,593 are distinct from those identified in AZT-resistant HIV-1 isolates from treated individuals (18).

Direct evidence that these amino acid changes gave rise to an L-693,593-resistant virus was obtained by constructing a recombinant HIV-1 strain containing the mutations. The construction used an infectious molecular clone of HIV-1, NL4-3 (1), and substituted the 1,009-bp *Msc*I-to-*Bsp*1286I fragment of either A17 or HXB2 RT (amino acids 25 to 359). A recombinant virus was generated in the CD4-expressing HeLa cell line HT4-6C (6) and amplified by cocultivation with FDA-H9 cells. Supernatants from persistently infected FDA-H9 cultures were used to determine the sensitivities of the resulting viruses in MT-4 cell culture. These experiments confirmed that the two mutations were responsible for the

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HXB2 PISPIETVPV KLKPGMDGPK VKQWPLTEEK IKALVEICTe MEKEGKISKI
A17  ---p------ ---------- ---------- ---------- ----------
B17  ---------- ---------- ---------- ---------- ----------

  51 GPENPYNTPV FAIKKKDSTK WRKLVDFREL NKRTQDFWEV QLGIPHPAGL
     ---------- ---------- ---------- ---------- ----------
     ---------- ---------- ---------- ---------- ----------

 101 KKKKSVTVLD VGDAYFSVPL DEDFRKYTAF TIPSINNETP GIRYQYNVLP
     --N------- ---------- ---------- ---------- ----------
     --N------- ---------- ---------- ---------- ----------

 151 QGWKGSPAIF QSSMTKILEP FRKQNPDIVI YQYMDDLYVG SDLEIGQHRT
     ---------- ---------- ---------- C--------- ----------
     ---------- ---------- ---------- C--------- ----------

 201 KIEELRQHLL RWGLTTPDKK HQKEPPFLWM GYELHPDKWT VQPIVLPEKD
     ---------- ---------- ---------- ---------- ----------
     ---------- ---------- Y--------- ---------- ----------

 251 SWTVNDIQKL VGKLNWASQI YPGIKVRQLC KLLRGTKALT EVIPLTEEAE
     ---------- ---------- ---------- --------1- ----------
     ---------- ---------- ---------- ---------- ----------

 301 LELAENREIL KEPVHGVYYD PSKDLIAEIQ KQGQGQWTYQ IYQEPFKNLK
     ---------- ---------- ---------- ---------- ----------
     ---------- ---------- ---------- ---------- ----------

 351 TGKYARMRGA HTNDVKQLTE AVQKITtESI VIWGKTPKFK lPIQKETWET
     ---------- ---------- ---------- ---------- ----------
     ---------- ---------- ---------- ---------- ----------

 401 WWTEYWQATW IPEWEFVNTP pLVKLWYQLE KEPIVGAETF YVDGAANRET
     ---------- ------v--- ---------- ---------- ----------
     ---------- ------v--- ---------- ---------- ----------

 451 KLGKAGYVTn RGRQkVVpLT nTTNQkTELQ AIYLALQDsG LEVNIVTDSQ
     ---------- K--------- ---------- ---------- ----------
     ---------- K--------- ---------- ---------- ----------

 501 YALGIIQAQP DQSESELVNQ IIEQLIKKEK VYLAWVPAHK GIGGNEQVDK
     ---------- -K--------- ---------- ---------- -i--------
     ---------- -K--------- ---------- ---------- ----------

 551 LVSAGIRKiL
     ----------
     ----------
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FIG. 3. Comparison of the deduced amino acid sequences of L-693,593-resistant RTs and wild-type HIV-1 HXB2 RT (24, 28). The deduced amino acid sequences of HXB2, A17, and B17 are indicated. Changes in A17 and B17 which also appear in another HIV-1 IIIb molecular clone, BH10 (24, 28), are indicated in lowercase letters in the HXB2 sequence only. Positions of sequence identity in A17 and B17 are indicated by dashes. Amino acid changes are in uppercase letters, and positions of silent nucleotide changes are in lowercase letters.

specific resistance observed in virus from the original biologically cloned A17 cell line (Table 2).

Cross-resistance to other nonnucleoside analog RT inhibitors. Finally, we wished to explore the question of cross-resistance to two recently described nonnucleoside inhibitors of HIV-1 RT, the tetrahydroimidazo[4,5,1-*jk*][1,4]benzodiazepin-2(1*H*)-thione (TIBO) compound R82150 (25) and the dipyridodiazepinone BI-RG-587 (22). These inhibitors are structurally unrelated to the pyridinone inhibitors, yet the three classes of compounds share many properties. These include the specificity for HIV-1 RT, the lack of kinetic competition with nucleoside triphosphates, and the pattern of template-primer preference (11, 22, 25). The recombinantly derived L-693,593-resistant A17 virus was highly cross-resistant to both structurally unrelated inhibitors R82150 and BI-RG-587 (Table 2). Similar results were obtained in enzymatic assays using the A17 RT expressed in *E. coli* (data not shown). These data suggest that these three structurally distinct RT inhibitors act via a common binding site and constitute a single pharmacologic class of RT inhibitor. This conclusion was independently obtained from experiments which demonstrate that both R82150 and BI-RG-587 are able to displace radiolabeled L-697,639 from the enzyme-template–primer-inhibitor complex (11).

DISCUSSION

In this report, we have described two mutations in HIV-1 RT; each mutation contributed partial resistance to HIV-1-specific pyridinone RT inhibitors. Two resistant viruses, A17 and B17, carried both mutations. We do not know whether resistance in these two viruses arose independently. Additional resistant viruses from passage 4 and from independent cultures will be examined to determine whether additional mutations exist which can give rise to viable L-693,593-resistant virus.

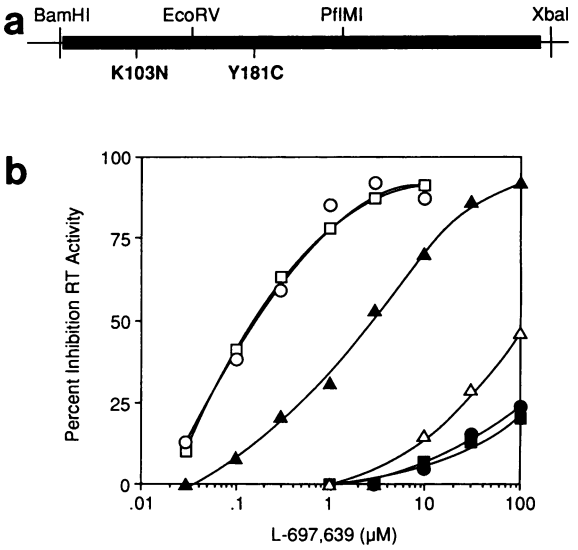

FIG. 4. Two amino acid changes contribute to L-693,593 resistance. (a) Diagram of restriction endonuclease sites used to exchange fragments between resistant and sensitive RTs. (b) Sensitivities of specific RTs to L-697,639. Symbols: □, pHRTRX2; ■, A17; ●, A17 *Bam*HI-*Pfl*MI; ○, A17 *Pfl*MI-*Xba*I; ▲, A17 *Bam*HI-*Eco*RV; △, A17 *Eco*RV-*Xba*I.

The identified mutations are located in regions of the RT which have been previously recognized as being important for enzyme activity. Our data suggest that these regions are in spatial proximity in the folded RT molecule. Both positions K-103 and Y-181 are invariant in HIV-1 isolates whose sequences are known (24). The more potent mutation (Y-181 to C) is located adjacent to the YG/MDD motif found in most DNA and RNA polymerases (2, 3, 16, 33). Mutations at Y-183, D-185, and D-186 have been shown to eliminate or severely reduce RT activity (19, 20). The less potent mutation (K-103 to N) lies adjacent to another region which is conserved in all RTs (amino acids 110 to 121; 3, 16). Mutations here have been shown to give rise to a variety of phenotypes, ranging from complete loss of enzymatic activ-

TABLE 2. Sensitivities of recombinant A17 and HXB2 viruses

Compound	CIC_{95}[a] (nM)		Fold resistance
	rA17	rHXB2	
L-696,040	200,000	200	1,000
L-697,639	100,000	50	2,000
AZT	25	25	1
Dideoxyinosine	50,000	25,000	2
TIBO R82150	100,000	100	1,000
BI-RG-587	100,000	100	1,000

[a] CIC_{95}, inhibitor concentration that reduced the spread of infection by at least 95%.

ity (D-110 to E) to altered sensitivities to phosphonoformate and AZT triphosphate (D-113 to E, A-114 to S, and Y-115 to N) (14, 19, 20). It was on the basis of these latter mutations that Larder et al. (20) proposed this region to be involved in PP_i exchange. Our finding raises the possibility that pyridinone inhibitors act through the PP_i site of RT. The observation that phosphonoformate is able to displace radiolabeled L-697,639 from RT complexes supports this hypothesis (11). The unchanged sensitivity of L-693,593-resistant virus to phosphonoformate could be accounted for by differences in the specific molecular contacts made by these compounds at this site. An alternative hypothesis, that the binding sites are distinct but communicate allosterically, cannot be excluded.

It is interesting that L-693,593-resistant viruses are fully cross-resistant to the structurally unrelated RT inhibitors TIBO and BI-RG-587. These three classes of HIV-1-specific RT inhibitors share many properties and appear to share a common binding site on RT. Analysis of viruses resistant to TIBO or BI-RG-587 may provide additional information to characterize this region of RT more extensively.

Our ability to isolate pyridinone inhibitor-resistant HIV-1 stands in sharp contrast to the reported inability to isolate in cell culture HIV-1 that is resistant to AZT (34; unpublished data). Although the virus can be successfully passaged in the presence of AZT, the resulting population remains sensitive to the inhibitor. We speculate that this difference derives from the fact that the nucleoside analog AZT, unlike the pyridinone inhibitors, is phosphorylated by host cells and interacts with host cell enzymes. Over time, the cell culture population adapts metabolically to minimize the toxic effects of the analog; this reduces the effective antiviral potency and permits spread of the infection through the culture. Thus, bona fide AZT-resistant viruses are not readily isolable in cell culture. In contrast, pyridinone inhibitors act directly and specifically on RT. The emergence of viral resistance is not obscured by the response of host cells, and isolation is facilitated. Studies are in progress to address this point.

L-697,639 and a related pyridinone inhibitor are currently in initial safety and tolerability studies in humans (11). It will be important to evaluate the potential for the emergence of resistant HIV-1 variants in the clinic. Development of resistance is a recognized consequence of long-term antimicrobial chemotherapy. Such has been the experience in the clinical use of AZT (17). Multiple mutations within the viral RT contribute to the high levels of resistance found in HIV-1 isolates from AZT-treated individuals (18). It remains to be seen whether resistance to pyridinone inhibitors, should it arise in clinics, will involve the specific amino acid changes identified in cell culture. It will also be of interest to determine whether clinical resistance to pyridinone inhibitors results in cross-resistance to the inhibitors TIBO and BI-RG-587.

Combination therapies comprising the use of HIV-1-specific RT inhibitors and nucleoside analog RT inhibitors, such as AZT and dideoxyinosine, will play an important role in minimizing the likelihood that drug-resistant strains of HIV-1 will emerge. These treatment approaches may also benefit from potential synergism between the antiviral effects of these mechanistically distinct inhibitors of RT (11, 30).

ACKNOWLEDGMENTS

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