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Characterization of a Human Immunodeficiency Virus Type 1 Variant with Reduced Sensitivity to an Aminodiol Protease Inhibitor

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Development of viral resistance to the aminodiol human immunodeficiency virus (HIV) protease inhibitor BMS 186,318 was studied by serial passage of HIV type 1 RF in MT-2 cells in the presence of increasing concentrations of compound. After 11 passages, an HIV variant that showed a 15-fold increase in 50% effective dose emerged. This HIV variant displays low-level cross-resistance to the C_2 symmetric inhibitor A-77003 but remains sensitive to the protease inhibitors Ro 31-8959 and SC52151. Genetic analysis of the protease gene from a drug-resistant variant revealed an Ala-to-Thr change at amino acid residue 71 (A71T) and a Val-to-Ala change at residue 82 (V82A). To determine the effects of these mutations on protease and virus drug susceptibility, recombinant protease and proviral HIV type 1 clones containing the single mutations A71T and V82A or double mutation A71T/V82A were constructed. Subsequent drug sensitivity assays on the mutant proteases and viruses indicated that the V82A substitution was responsible for most of the resistance observed. Further genotypic analysis of the protease genes from earlier passages of virus indicated that the A71T mutation emerged prior to the V82A change. Finally, the level of resistance did not increase following continued passage in increasing concentrations of drug, and the resistant virus retained its drug susceptibility phenotype 34 days after drug withdrawal.

Current human immunodeficiency virus type 1 (HIV-1) therapeutic agents are limited to inhibitors of reverse transcriptase (RT). However, viruses with reduced sensitivity to these compounds have been isolated in vitro as well as from patients undergoing clinical treatment (10, 20, 21, 28, 32). HIV-1 protease is responsible for the specific cleavage of both Gag and Gag-Pol polyproteins and is also required for the assembly and maturation of infectious virions; hence, it is another important anti-HIV-1 target (5, 13, 24). To date, a number of substrate-based inhibitors, Ro 31-8959, A-77003, A-80987, SC52151, L-735,524, P9941, and XM323 (3, 9, 15, 17, 19, 27, 29, 34), have been described. These compounds are active against both acute and chronic HIV-1 infections, a property which distinguishes them from RT inhibitors (5, 13, 24). Clinical efficacy of protease inhibitors is currently under investigation. Development of viral resistance to various protease inhibitors has been demonstrated in vitro by serial passage of HIV-1 in the presence of increasing concentrations of drug. Specific resistance mutations within the protease gene have been reported for at least six protease inhibitors: Ro 31-8959 (Gly to Val at residue 48 and Leu to Met at residue 90 [G48V/L90M]), A-77003 (Arg to Gln at residue 8 and Met to Ile at residue 46 [R8Q/M46I]; Met to Phe/Leu at residue 46 [M46F/L]; Val to Ile at residue 32 and Val to Ile at residue 82 [V32I/V82I]), A-80987 (Val to Ile at residue 32, Met to Leu at residue 32, and Ile to Val at residue 47 [V32I/M46L/I47V]), P9941 (Val to Ala at residue 82 [V82A]), XM323 (Val to Phe at residue 82 and Leu to Val at residue 97 [V82F/L97V]), and RPI312 (Ile to Val at residue 84 [I84V]) (4, 6, 7, 12, 14, 16, 25, 26, 35).

BMS 186,318 is a novel aminodiol-based HIV-1 protease inhibitor exhibiting potent antiviral activity against HIV-1, HIV-2, and simian immunodeficiency virus with a 50% effective dose (ED_{50}) of 0.02 to 0.1 μM and a therapeutic index of >1,000 (1, 2). The compound has been shown to inhibit the production of infectious virus particles from chronically infected cells and to block the processing of HIV coat protein precursor (2). To study the potential of resistance development and to characterize the amino acids of the HIV-1 protease which interact with BMS 186,318, we attempted to isolate an HIV-1 variant with reduced sensitivity by serial passage in vitro. In this study, we have identified and characterized the phenotype and genotype of one such variant.

MATERIALS AND METHODS

Cells and viruses. The CEM-SS human T-cell line, HeLa-CD4$^+$ clone 1022 cell line, MT-2 cell line, RF strain of HIV-1, and pNL4-3 proviral clone were obtained through the AIDS Research and Reference Reagent Program, Division of AIDS, National Institute of Allergy and Infectious Diseases, and were originally contributed by P. Nara, B. Chesebro, D. Richman, M. Martin, and R. Gallo.

Antiviral agents. The protease inhibitors BMS 186,318, Ro 31-8959, A-77003, and SC52151 were synthesized at Bristol-Myers Squibb. Zidovudine was purchased from Burroughs Wellcome (Research Triangle Park, N.C.).

Passage of HIV-1 RF in MT-2 cells. MT-2 cells (5×10^5/ml) were infected with HIV-1 RF at a multiplicity of infection (MOI) of 0.1. Following a 1-h adsorption period, inoculum was removed by washing and the cells were resuspended in RPMI containing BMS 186,318. When cultures exhibited significant microscopic cytopathogenicity, the culture supernatants were removed by centrifugation, and 0.5 to 1.0 ml was used to infect fresh MT-2 cells as described. The concentration of BMS 186,318 was increased gradually during passage. Cell pellets were washed once with phosphate-buffered saline and frozen at −70°C for DNA sequence analysis.

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Drug susceptibility assay. MT-2 cells (5×10^5 cells per ml) were infected with equal amounts of the appropriate viruses (MOI of 0.001). After a 1- to 2-h adsorption period, cells were washed and dispensed in duplicate into 96-well plates containing half-log dilutions of the appropriate drug. Three days after infection, culture supernatants were collected and the levels of p24 were determined by HIV-1 p24 enzyme-linked immunosorbent assay (NEN Research Products, Dupont, Wilmington, Del.). Data are expressed as (p24 level in the presence of drug/absence of drug) $\times$ 100 and are plotted against log drug concentration (micromolar). The ED_{50} was calculated by extrapolation from the curve.

DNA sequencing of the protease genes. Total cellular DNA was prepared by resuspension of 2×10^6 infected cells in 1 ml of lysis buffer (50 mM KCl, 10 mM Tris-HCl [pH 8.3], 2.5 mM $MgCl_2$, 0.45% Nonidet P-40, 100 μg of proteinase K per ml) and incubation at 55°C for 1 h to lyse the cells and then 95°C for 15 min to inactivate the proteinase K. The protease-coding sequence was amplified by PCR using 20 μl of the total cellular DNA extract and primers 5′-GAA TTC TCT AGA CAA GGA ACT GTA TC-3′ (BR13) and 5′-CTG GTA CAG TAT CGA TAG GAC TAA TG-3′ (BR10) for the 5′ and 3′ ends, respectively, using standard PCR conditions (30). The PCR fragments were then digested with *Cla*I and *Xba*I, ligated with *Cla*I- and *Xba*I-digested pBluescript II KS+ (Stratagene, Inc., La Jolla, Calif.), and transformed into *Escherichia coli* DH5 competent cells (BRL Life Technologies, Gaithersburg, Md.). DNA was prepared from recombinant clones, and the DNA sequence was determined by the dideoxy-chain termination method using T3 and T7 primers and a Sequenase version 2.0 DNA sequencing kit (United States Biochemical, Cleveland, Ohio) (33).

Construction of recombinant HIV-1 variants. To create HIV-1 strains with defined mutations in the protease gene, we constructed a pNL4-3 proviral clone (pNL4-3-PXC) having the protease gene flanked by *Xba*I and *Cla*I sites to allow easy substitution of the desired protease genes. This pNL4-3 proviral clone was constructed by first cloning a 3,733-bp *Apa*I (2011)-to-*Eco*RI (5744) fragment from pNL4-3 into pBluescript II SK+, creating plasmid pBS-43AE. Plasmid pBS-43XC was created by changing bases 2223 to 2224 and bases 2564 to 2568 from GA to TC and TGAG to CGAT, respectively, by site-directed mutagenesis using primers 5′-GCA GGA GCC TCT AGA CAA G-3′ and 5′-CAT TAG TCC TAT CGA TAC TGT ACC AG-3′ and the method of Kunkel (18). To generate pNL4-3-PXC, the 1,481-bp *Apa*I-to-*Age*I fragment of pNL4-3 was replaced with the *Apa*I-to-*Age*I fragment from pBS-43-XC. To introduce defined mutations in the protease gene of pNL4-3-PXC, the protease gene from pNL4-3 was PCR amplified by using primers BR10 and BR13 and cloned into pBluescript II KS+, creating plasmid pBS-PXC. Mutations were created by site-directed mutagenesis as described above, using primers A71T (5′-GGA CAT AAA ACT ATA GGT ACA G-3′) and V82A (5′-CTA CAC CTG CCA ACA TAA TTG G-3′) with pBS-PXC single-stranded DNA, to create plasmids having mutation A71T, V82A, or A71T/V82A in the protease gene. To create pNL4-3 proviral clones having mutations A71T, V82A, and A71T/V82A, the *Xba*I-to-*Cla*I fragment of pNL4-3-PXC was replaced with the appropriate *Xba*I-to-*Cla*I fragment from the pBS-PXC clones. The presence of each of the mutations in the pNL4-3 proviral clones was verified by DNA sequence analysis.

To prepare the recombinant mutant virus, HeLa-$CD4^+$ clone 1022 cells in 100-mm-diameter petri dishes were transfected with mutant proviral DNA by a calcium phosphate technique (Stratagene). Culture supernatant containing infectious virus was harvested 4 to 6 days after transfection and was further expanded in MT-2 cells to produce working virus stocks. Infectious titers were determined by plaque assay in HeLa-$CD4^+$ cells.

Expression of mutant HIV-1 protease in *E. coli*. HIV-1 protease was expressed in *E. coli* as a maltose-binding protein–protease fusion protein. The protease-coding regions from the pBS-PXC clones having the A71T, V82A, and A71T/V82A mutations were PCR amplified by using primers 5′-CAC AGA GCT CCA TCG AGG GAA GAG GAG CCG ATA GAC AAC GAA CTG-3′ and 5′-GCG AAT TCA AAA ATT TAA AGT GCA ACC-3′ for the 5′ and 3′ ends, respectively. The PCR products were digested with *Sst*I and *Eco*RI, ligated with *Sst*I- and *Eco*RI-digested pMAL-C2 (New England Biolabs, Inc., Beverly, Mass.), and transformed into *E. coli* JM109 competent cells.

Purification of HIV-1 protease. Protease expression clones were grown to an optical density at 600 nm of 0.5 and then induced with 1 mM isopropylthiogalactopyranoside (IPTG) for 90 min. Proteases were purified as described previously (23). Briefly, cell pellets from 2-liter cultures were resuspended in 50 ml of lysis buffer (50 mM Tris-HCl [pH 6.5], 30 mM NaCl, 1 mM EDTA, 1 mM dithiothreitol [DTT], 0.5% Nonidet P-40, 1 mM phenylmethylsulfonyl fluoride) and sonicated. The suspension was centrifuged for 30 min at 9,500 $\times$ *g*. The supernatant was diluted fourfold with column loading buffer (10 mM sodium phosphate [pH 6.5], 30 mM NaCl, 0.25% Tween 20, 1 mM DTT) and loaded onto a 2-ml amylose resin column (New England Biolabs, Inc.) at 4°C. The column was washed with 5 volumes of column loading buffer and 10 volumes of wash buffer (10 mM sodium phosphate [pH 6.5], 0.5 M NaCl, 1 mM DTT, 1 mM EDTA). Fusion protein was eluted with wash buffer containing 10 mM maltose. Fractions were assayed for protein by the Coomassie blue protein assay (Pierce Chemical Co., Rockford, Ill.). All fractions containing more than 100 μg of protein per ml were pooled and diluted 1:1 with 10 M urea in phosphate buffer (100 mM sodium phosphate [pH 6.5], 60 mM NaCl, 20 mM DTT, 2 mM EDTA). The protein was dialyzed against 1 liter of decreasing amounts of urea (4, 2, 1, 0.5, and 0.25 M) in 50 mM $NaPO_4$–30 mM NaCl–1 mM DTT–1 mM EDTA (pH 6.5) for 45 to 60 min. Dialysis was then performed against a phosphate buffer containing 25 mM sodium phosphate (pH 6.5), 1 mM DTT, 1 mM EDTA, and 5% glycerol for 1 h and then dialyzed against 2 liters of 50 mM morpholineethanesulfonic acid (MES; pH 6.5)–1 mM EDTA–1 mM DTT–5% glycerol (buffer A) for 3 to 4 h. Protease was separated from maltose-binding protein on a Mono S (Pharmacia Biotech, Piscataway, N.J.) column, using a gradient of 0 to 1 M NaCl in buffer A. Wild-type protease generally elutes at 0.5 NaCl and is 99% pure; the mutants eluted around 0.2 M NaCl.

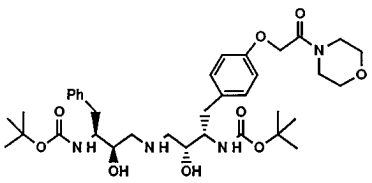

FIG. 1. Structure of BMS 186,318.

Assay for protease activity. Sensitivity of HIV-1 protease to protease inhibitors was determined by a peptide substrate cleavage assay. The inhibitor was diluted in reaction buffer consisting of 50 mM sodium acetate and 1 mg of bovine serum albumin per ml (pH 5.5). A 20-μl aliquot of inhibitor solution was mixed with a 20-μl aliquot of peptide substrate H_2N-Val-Ser-Gln-Asn-(-β-naphthylalanine)-Pro-Ile-Val-COOH (final substrate concentration of 0.284 μM). The assay was initiated with purified enzyme (20 μl) diluted with reaction buffer to a final concentration of 1 to 10 nM. After 30 min at 37°C, reactions were quenched with 150 μl of 5% aqueous phosphoric acid. Protease products were analyzed on a reverse-phase high-pressure liquid chromatograph by a variation of the method of Heimbach et al. (11). Inhibition constants (K_i) were determined by measuring rates of substrate to product conversion at six inhibitor concentrations as previously described (31).

RESULTS

Isolation of resistant virus. The aminodiol inhibitors represent a novel structural class of HIV-1 protease inhibitors (1, 2). Studies were initiated to determine the frequency and degree of resistance development following passage of HIV-1 in the presence of the aminodiol inhibitor BMS 186,318 (Fig. 1). MT-2 cells were infected with HIV-1 RF at an MOI of 0.1 and cultured in the presence of 0.3 μM ($3 \times ED_{50}$) BMS 186,318. The concentration required to inhibit virus replication by 90% was approximately 0.3 to 0.5 μM as measured by a cell protection-XTT dye reduction assay (36). When virus-induced cytopathic effect was evident, the culture supernatant was harvested and used to inoculate fresh MT-2 cells. The drug concentration and duration of treatment for each passage are summarized in Table 1.

Drug susceptibility testing was performed on culture super-

TABLE 1. Isolation of BMS 186,318-resistant HIV-1 RF

Passage no.	Concn (μM)	Days between passages	ED_{50} (μM)
1	0.3	2	0.04 (1)[a]
2	0.4	4	
3	0.4	6	0.10 (3)
4	0.8	5	
5	0.8	6	
6	1.0	6	
7	1.2	3	
8	1.2	6	0.10 (3)
9	1.4	7	
10	1.4	5	
11	1.4	3	0.58 (15)
12	3.0	4	
13	6.0	7	
14	10	7	
15	10	10	
16	10	5	
17	10	7	0.61 (15)

[a] Numbers in parentheses indicate fold increase.

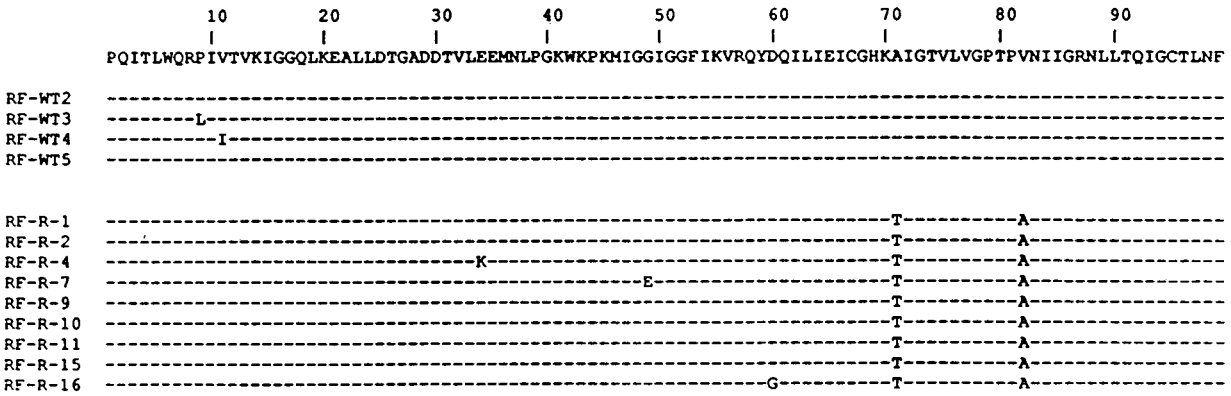

FIG. 2. Amino acid sequence comparison of protease gene clones from the wild type (RF-WT) and the BMS 186,318-resistant variant (RF-R). Dashes indicate sequence homology.

natants derived from passages 1, 3, 8, 11, and 17. A significant reduction in the level of susceptibility to BMS 186,318 was first observed at passage 11, with a 15-fold increase in ED_{50}. Continued passage of the BMS 186,318-resistant virus in the presence of increasing concentrations (10 μM) of compound did not result in an increased level of resistance as determined by susceptibility assays. The ability of virus to grow at high concentrations of the compound at later passages was most likely the result of high-MOI passage, since the virus titer was not determined between passages. To study the stability of the BMS 186,318-resistant phenotype, HIV-1 RF from passage 13 was cultured in the absence of drug for seven additional passages, representing 34 days. Results indicated that virus from this later passage maintained the same level of resistance to BMS 186,318 despite removal of drug (data not shown).

Identification of resistance mutations. To identify the amino acid changes responsible for resistance to BMS 186,318, the protease genes from BMS 186,318-resistant and wild-type HIV-1 RF strains were PCR amplified and cloned. DNA sequencing of wild-type and resistant clones showed that all nine of the resistant clones contained A71T and V82A changes, while none of the four wild-type clones contained these changes (Fig. 2). In addition, isolated alterations resulting in a Glu-to-Lys change at residue 34, a Gly-to-Glu change at residue 49 and an Asp-to-Gly change at residue 60 were also observed. Since these changes were not consistently observed, they may represent random changes that had occurred during passage of virus and/or are the result of PCR amplification.

To determine whether the A71T or the V82A substitution confers the drug-resistant phenotype, pNL4-3 proviral HIV-1 clones containing the single protease mutations A71T and V82A or the double substitution A71T/V82A were constructed. Recombinant virus containing either the V82A change or both A71T and V82A changes were resistant to BMS 186,318 (Table 2). In contrast, a significant change in sensitivity to BMS 186,318 could not be detected for the virus containing the A71T mutation alone. All of the recombinant viruses remained sensitive to zidovudine.

To determine whether the A71T or V82A mutation occurred first, virus from two earlier passages were also PCR amplified and sequenced. As the data in Table 3 illustrate, 25% of the clones sequenced from passage 4 virus contained the A71T mutation whereas the remaining clones were genotypically wild type. Results from clones derived from passage 8 virus indicated that 53% of the clones contained only the A71T mutation, none of the clones contained the V82A mutation, and 27% of the clones now contained both A71T and V82A mutations. By passage 11, all clones contained both A71T and V82A changes (Fig. 2; Table 3). These results indicate that the A71T change occurred earlier than had the V82A mutation. Moreover, the passage 8 virus exhibited minimal resistance despite the fact that 27% of the viral population contained the A71T and V82A mutations. This could be due to the sensitivity limitation of the viral drug susceptibility assay.

Characterization of resistant proteases. *E. coli* clones which expressed HIV-1 proteases containing either the A71T, V82A, or A71T/V82A mutation in the NL4-3 genetic background were constructed. The purified enzymes from these clones were then examined for the ability to cleave a modified Gag peptide substrate; the biochemical properties of these purified enzymes are summarized in Table 4. The V82A substitution reduced the substrate affinity (K_m) approximately twofold and decreased the turnover rate (k_{cat}) of protease by fourfold. Interestingly, the double mutant A71T/V82A had a near normal K_m but had a significantly reduced (sevenfold) k_{cat} value. The A71T substitution had no significant effect on the K_m, k_{cat}, and k_{cat}/K_m values, suggesting that the A71T change has no effect on the proteolytic cleavage of this substrate. The inhibition constant of BMS 186,318 for each of these purified enzymes was also determined (Table 4). Results confirmed the virology data and show that both the A71T/V82A and V82A substitutions dramatically reduce the affinity of HIV-1 protease for BMS 186,318. The A71T mutation alone, as expected, had little effect on the K_i of BMS 186,318.

Lack of cross-resistance to other protease inhibitors. HIV-1 protease mutations conferring resistance to other protease in-

TABLE 2. Drug sensitivities of recombinant viruses with protease mutations

Inhibitor	ED_{50} (μM) [fold]			
	HIV-1 NL4-3	A71T/V82A	V82A	A71T
BMS 186,318	0.032	0.26 [8]	0.13 [4]	0.04
Zidovudine	0.002	0.004	0.002	0.002

TABLE 3. Appearance of protease mutations in HIV-1 RF

Passage no.	Fraction (%) of clones containing protease mutation(s)			
	Wild type	A71T	V82A	A71T + V82A
4	12/16 (75)	4/16 (25)	0	0
8	3/15 (20)	8/15 (53)	0	4/15 (27)
11	0	0	0	9/9 (100)

TABLE 4. Biochemical properties of protease mutants

Genotype	K_m (μM)	k_{cat} (min^{-1})	k_{cat}/K_m (μM^{-1} min^{-1})	BMS 186,318 k_i (nM)
Wild type	1,010	6,400	6.3	34
A71T	820	5,600	6.8	59 (2)[a]
V82A	1,800	1,600	0.9	1,080 (32)
A71T/V82A	800	860	1.1	1,140 (34)

[a] Numbers in parentheses indicate fold change.

hibitors have recently been described (4, 6, 7, 12, 14, 16, 25, 26, 35). Drug sensitivity studies were conducted to determine if BMS 186,318-resistant virus and recombinant protease enzymes remained sensitive to other protease inhibitors. As Table 5 illustrates, the BMS 186,318-resistant virus showed some (fourfold) cross-resistance to A-77003 but remained susceptible to Ro 31-8959 and SC52151. Similar results were obtained with recombinant proteases (Table 5). Enzymes containing the V82A or A71T/V82A mutation remained sensitive to Ro 31-8959 and SC52151 and showed a low level of resistance (four- to fivefold) to A-77003. Furthermore, results from enzyme assays confirmed that the V82A substitution was responsible for resistance to BMS 186,318 and A-77003, while the A71T alteration had no effect on drug sensitivity. These results suggest that the V82A mutation is the key change required to confer resistance to this protease inhibitor.

DISCUSSION

Most HIV-1 therapeutic agents described to date involve inhibition of reverse transcription and are effective against acute but not chronic infections. Moreover, the adverse side effects associated with nucleoside inhibitors coupled with the emergence of resistant virus following drug treatment indicate a need for alternative therapies. HIV-1 protease inhibitors are distinguished from RT inhibitors in their ability to block viral spread during chronic infection.

We have previously identified and described a group of aminodiol compounds representing a novel structural class of peptide mimetics that are potent and selective inhibitors of HIV-1 protease (1, 2). To understand the potential for selecting viruses with reduced sensitivity to these aminodiol inhibitors, we cultured HIV-1 RF in the presence of increasing concentrations of BMS 186,318, a representative compound in this class. We selected an HIV-1 variant that displayed a moderate reduction in its level of sensitivity (15-fold) to this compound (Table 1). Both the length of time (53 days, 11 passages) and moderate degree of sensitivity change are similar to those reported for HIV-1 variants selected by other protease inhibitors (4, 6, 7, 12, 14, 16, 25, 26, 35). Genetic analyses of the resistant variant identified two common amino acid changes at residues 71 and 82 of the protease (Fig. 2). Biochemical analyses (Table 4) of recombinant protease revealed that although the enzyme contains both A71T and V82A mutations, only the V82A substitution affects the drug sensitivity of protease (32- to 34-fold increase in K_i). The slight cross-resistance (fivefold) of the V82A mutant to the C_2-symmetric inhibitor A-77003 may be due to the low level of structural similarity shared by both inhibitors. Drug sensitivity testing of the recombinant viruses containing these mutations also confirmed the role of the V82A mutation in resistance development (Table 2). The correlation of drug resistance by a mutant protease derived from a HIV-1 variant contrasts to that observed in zidovudine resistance; in this case, the RT from zidovudine-resistant viruses exhibits wild-type drug sensitivity (20, 21, 28).

Recent reports indicate that the V82A, V82I, and V82F changes in the protease gene are associated with decreased sensitivity to several other protease inhibitors, including P9941, A-77003, and XM323 (16, 25, 26). These results are not surprising because the region of HIV-1 protease from residues 78 to 88 is highly conserved (8), suggesting a functional importance for this domain. Furthermore, in an extensive mutagenesis study of the HIV-1 protease, Loeb et al. (22) showed that amino acid 82 is located in a region sensitive to mutation and presumably represents a major structural and functional determinant of the protease. Indeed, it has been described that the V82A alteration results in a protease with reduced activity (22, 26). This observation is consistent with our data which showed that the V82A protease has k_{cat} and k_{cat}/K_m values lower than those of the wild-type enzyme (Table 4). In contrast, the A71T mutation did not appear to significantly affect either the substrate affinity (K_m) or the catalytic cleavage (k_{cat}). The combination of A71T and V82A mutations restored the K_m to wild-type levels with only a sevenfold reduction in k_{cat}, indicating that the double mutant may be able to bind substrate well at the expense of slower cleavage.

Molecular modeling of BMS 186,318 with HIV-1 protease suggests that the phenyl and *t*-butoxycarbonyl groups occupy the S_1 and S_2 binding sites, respectively, while the morpholino group is mostly exposed to the solvent (unpublished results). Specifically, the phenyl groups of the bound inhibitor make contact with residues 49, 50, 81, 82, and 84 at each of the enzyme subunits. The reduced drug sensitivity resulting from the V82A mutation in the S_1 subsite may be due to decreased hydrophobic interaction between the smaller Ala at positions 82 and 82′ and the corresponding phenyl group of the inhibitor. Interestingly, residue 71 is outside the enzyme active site and does not come into direct contact with the inhibitor.

Since BMS 186,318 induced a moderate level of viral resistance (15-fold), and more importantly the resulting virus remained sensitive to a variety of structurally unique inhibitors

TABLE 5. Cross-resistance of mutant viruses and protease

Inhibitor	HIV-1 RF ED_{50} (μM)		Enzyme K_i (nM)			
	Wild type	Resistant[a]	Wild-type HIV-1 NL4-3	A71T	V82A	A71T + V82A
BMS 186,318	0.03	0.44 (15)[b]	34	59 (2)	1,080 (32)	1,140 (31)
A-77003	0.04	0.14 (4)	1.2	1.4	6.1 (5)	4.9 (4)
Ro 31-8959	0.002	0.004 (2)	0.98	0.7	2.8	1.5
SC52151	0.01	0.02 (2)	2.3	2.2	2.2	3.4
Zidovudine	0.003	0.003	ND[c]	ND	ND	ND

[a] Passage 11 virus.
[b] Numbers in parentheses indicate fold change.
[c] ND, not determined.

currently in development (Ro 31-8959 and SC52151) (Table 5), it may be possible to overcome drug resistance by either increasing the treatment dose or switching to other drugs. Finally, it is encouraging that various clinical candidate protease inhibitors induce different sets of amino acid substitutions in protease (4, 6, 7, 12, 14, 16, 25, 26, 35), suggesting that the combination of two protease inhibitors may represent an effective strategy against HIV-1.

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