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The I84A and I84C Mutations in Protease Confer High Level Resistance to PIs and Impair Replication Capacity

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INTRODUCTION

Resistance to protease inhibitors (PIs) is attributed to the development of mutations both within and outside of the HIV protease active site as well as the *gag* cleavage sites. Mutations within the active site (primary mutations) significantly reduce the binding affinity of the inhibitors to the mutant protease, and thus play a critical role in conferring resistance. However, mutations outside of the active site and at the cleavage sites may in some cases contribute to resistance by restoring the fitness of the mutant virus rather than by direct diminution of drug binding. The I84V mutation is an important primary mutation associated with resistance to the protease inhibitor class and has been observed in patients following treatment with currently available PIs. An alanine mutation at position 84 (I84A) has been observed in variants following *in vitro* passage with the experimental PI BILA 1906 BS. However, the clinical relevance of the I84A and other I84 mutations is not well characterized. We have observed both I84A and I84C mutations in patient-derived viruses following treatment with PIs. The aim of the present study is to investigate the PI susceptibility and replication capacity (RC) of I84A and I84C mutant variants.

OBJECTIVES

- To assess the role of the I84A and I84C mutations in the development of high-level resistance to PIs.
- To evaluate the mechanism by which the I84A and I84C and accompanying *gag* cleavage site mutations contribute to resistance and RC.

MATERIAL AND METHODS

- Longitudinal plasma samples were obtained from one subject who was receiving RTV/SQV dual PI therapy.
- The HIV-1 protease coding region and gag p7/p1 and p1/p6 cleavage sites were amplified from the plasma RNA by RT-PCR and sequenced by automated sequencer (ABI-373).
- Phenotypic susceptibility and RC was evaluated by Virologic, Inc. (PhenoSense HIV™).
- Molecular modeling studies were carried out with Insight II Software (Accelrys, San Diego).

RESULTS

- The I84C and I84A mutations were each identified in 8 separate isolates. The V82I primary mutation was observed in 3/8 isolates that had the I84C mutation. Secondary mutations at positions 10, 46, 47, 54, 63, 71 were commonly found along with the I84 mutations (Table 1). The susceptibility to PIs of these isolates were compared to analogous isolates containing the I84V mutation, with or without the I54V mutation.
- The median number of PI mutations were similar among different groups of isolates (4.5 to 5.5) except the I84V plus I54V isolates, which contained a higher number of mutations (median 7.5)
- Mutations at p7/p1 and/or p1/p6 cleavage sites (A431V and/or L449F/Q, respectively) were common in I84A (6/6), I84C (5/8) and I84V (5/6) containing I54V mutation, but uncommon in I84V isolates lacking the I54V mutation (1/6).
- Compared to I84V isolates lacking the I54V mutation (median, 3-fold), I84C isolates displayed substantially higher resistance to NFV (109-fold) and SQV (median, 48-fold) but similar susceptibility to LPV (Table 1, Figure 2).

RESULTS *continued*

- I84A isolates exhibited >30-fold median resistance to all of the PIs tested except LPV (Table 1, Figure 2).
- Compared to I84V isolates lacking I54V (median 3-fold), the I84V isolates with I54V displayed a significantly higher level of resistance to all PIs tested (median >22). In addition, these isolates were especially resistant to LPV (median 80-fold).
- The RC was impaired in all I84A and I84C mutant isolates: median RC values for I84A and I84C were 6.4% and 11%, respectively compared to 28% and 36% for I84V with/and without I54V, respectively (Figure 3).
- In the one subject with longitudinal data (Subject A5), the amino acid at position 84 evolved from wild type isoleucine (I) to valine (V), and subsequently to alanine (A) following viral rebound on RTV/SQV therapy (Figure 1), suggesting that the I84V mutant was the precursor to I84A.

Figure 1. Viral Load and the Genotypic Changes in Isolates from Subject A5

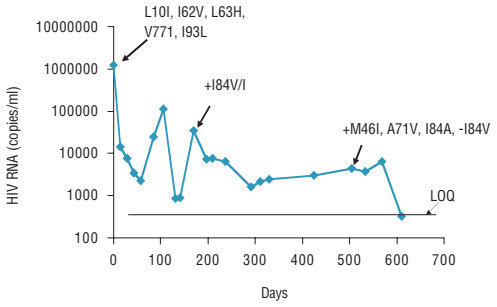
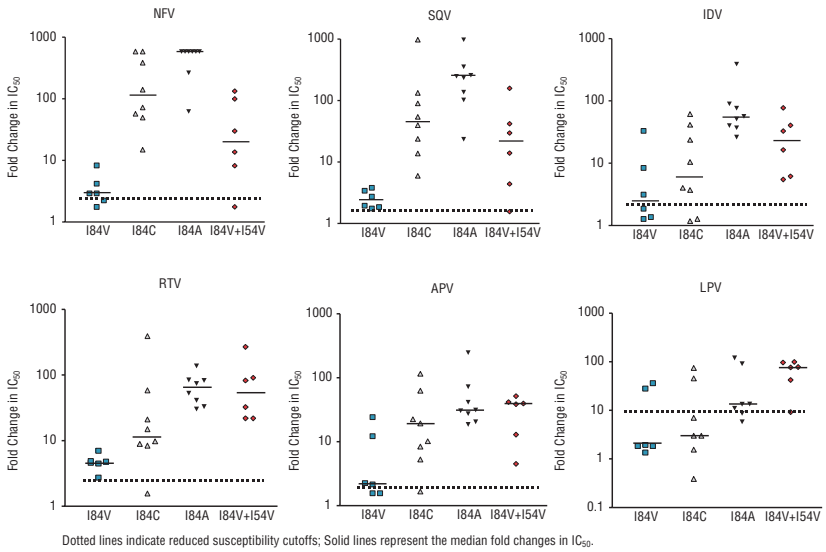


Table 1. Genotype, Phenotype and Replication Capacity (RC) of Resistant Isolates

Isolate	aa at 84	Mutations(s) in Protease* Other Mutations	Mutation in Cleavage Sites p7/p1	p1/p6	Fold Change (FC) in IC_{50} Compared to wt # NFV	SQV	IDV	RTV	APV	LPV	RC (% of WT)
C1	I84C		WT	WT	15.3	6.1	1.2	1.6	1.7	0.4	12.0
C2	I84C	K201, M361, V821	WT	S451N	58.7	14.1	1.3	8.6	5.4	NA	11.0
C3	I84C	L101, L19L/I, L241, L63P	WT	L449L/F	74.3	55.9	4.1	10.0	8.6	1.6	8.9
C4	I84C	L10F, L19L/V, L24I, M46L, L63P	A431V	L449F	395.3	92	10.7	9.1	10.4	3.1	0.6
C5	I84C	L10L/I, K20R, M36I, M46L, L63P, V82IV/I	WT	WT	50.7	40.8	3.8	15.3	19.8	3.1	15.0
C6	I84C	L10I, G16A, M46I, L63P, L76V	A431V	WT	143.8	24.4	62.9	21.6	64.1	46.8	13.0
C7	I84C	L10I, L24I, M46L, L63P, A71T, G73S, V771	A431A/V	L449F	600.0	1000	42.3	59.8	23.0	7.2	1.2
C8	I84C	L10I, K20R, L24I, M36V, M46L, L63P, V771, V82F	A431A	WT	600.0	137.2	24.2	400.0	118.1	77.0	2.2
Median					109.0	48.4	7.4	12.7	15.1	3.1	11.0
A1	184A	L33F, M46I, I63P	NA	NA	600.0	140.6	26.8	84.5	31.1	NA	NA
A2	184A	L10I, M46L, L63T, A71V	NA	NA	600.0	364.6	78.3	76.0	42.5	11.4	22.0
A3	I84A	L10F, M46I, L63P, A71V	WT	L449F	600.0	241.1	57.8	42.1	32.0	13.8	2.5
A4	I84A	L10V, M46I, L83A/P, A71V	A431V	L449Q	600.0	256.2	52.6	54.2	28.1	9.0	18.0
A5	I84A	L10I, M46I, L63H, A71V, V771	A431V	WT	271.0	105.0	41.0	31.0	19.0	6.0	NA
A6	I84A	L10I, M46I, L63P, A71V, L76V	A431V	WT	63.9	24.0	38.0	33.7	20.9	13.9	1.0
A7	I84A	L10F, L19I, M46I, I47V, I54V, L63P, A71V	A431V	S451I	600.0	1000	92.1	141.1	74.0	123.5	NA
A8	I84A	L10V, K20I, M36I, M46I, A71V, G73S, L76V	A431V	WT	600.0	265.5	400.0	86.2	252.2	94.4	6.4
Median					600.0	248.8	55.2	65.1	31.5	13.8	6.4
V1	I84V	L63P, G73S	WT	WT	3.0	3.9	1.3	4.9	1.6	1.9	24.5
V2	I84V	L10I, M46I, L76V	A431V	WT	3.0	2.0	8.6	7.2	24.8	37.5	14.9
V3	I84V	L10I, L63P, A71T, I85V	WT	WT	1.8	2.8	1.9	4.7	2.3	1.4	34.0
V4	I84V	L10I, M46L, L63C, V77I, P79A/S	WT	WT	4.3	1.8	3.2	2.8	1.6	1.9	31.1
V5	I84V	L10I, G16G/E, K20K/T, M36M/I, L63L/H	WT	WT	2.3	1.9	1.4	4.6	2.2	2.0	97.4
V6	I84V	L10V, K20I, M36I, M46I, L63P, A71V, L76V	WT	WT	8.5	3.5	33.9	5.0	12.5	28.9	18.3
Median					3.0	2.4	2.5	4.8	2.2	2.0	27.8
V7	I84V	L10I, M46I, 147V, I54V, L63P, A71T	A431V	WT	30.8	30.4	33.6	92.9	42.6	80.2	50.9
V8	I84V	L10F, M46I, I47V, I54V, L63P, A71T, P79N	A431V	WT	14.0	14.3	16.7	33.3	40.4	79.0	6.0
V9	I84V	L10I, L33F, M46M/I, I54V, L63P, A71T, L76V	A431V	WT	1.8	1.6	5.6	22.4	13.2	43.4	9.9
V10	I84V	L10L/I, K20I, M46I, I54V, L63P, A71A/T, V771, I85V	WT	WT	8.4	4.5	6.3	22.4	4.6	9.5	96.3
V11	I84V	L10I, K20T, M46I, I54V, L63A, A71T, V77I	A431V	WT	101.9	43.1	79.2	84.5	39.5	99.3	114.5
V12	I84V	L10I, K20R, L24I, L33F, M36I, M46I, I54V, L63P, A71T, G73S, V82I	A431V	S451N	136.6	162.3	41.5	275.0	52.7	102.7	20.0
Median					22.4	22.4	25.1	58.9	39.9	79.6	35.5

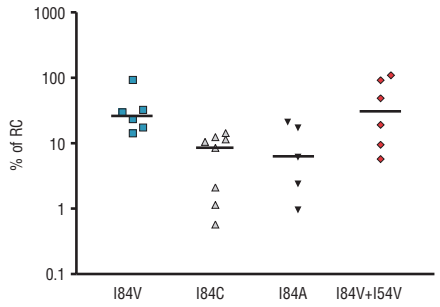
*Polymorphisms at the following positions not listed: 12, 13, 14, 15, 18, 33 (other than L33F), 35, 37, 39, 41, 43, 45, 57, 60, 61, 62, 64, 65, 66, 67, 69, 70, 72, 74, 83, 89, 91, 93, 95. NA, not available. NFV, nelfinavir; SQV, saquinavir; IDV, indinavir; RTV, ritonavir; APV, amprenavir, LPV, lopinavir. # the current reduced susceptibility cutoffs are 2.5 for RTV and NFV, 1.7 for SQV, 2.1 for IDV, 2.0 for APV, and 10 for LPV; For all PIs except LPV, purple color highlighted the FC values between cutoffs and 10, blue color for 10-50, red for > 50; For LPV, purple for 10-40, blue for >40.

Figure 2. Susceptibility of I84V, C and A Mutant Isolates to PIs



Dotted lines indicate reduced susceptibility cutoffs; Solid lines represent the median fold changes in IC_{50}.

Figure 3. Comparison of Replication Capacity of the I84V, C and A Mutant Isolates



Structural Analysis of Mutant Proteases

- The amino acid at position 84 lies within the active site of HIV protease (Figure 4). Since HIV protease is a homodimer, there are two 84 residues in each active site (Ile 84 and Ile 184).
- The I84 and I184 residues contact the PIs (RTV in this example) at the symmetry-related P2/P1' groups (Figure 4).
- Table 2 shows the increase in surface area created within the active site due to the suboptimal fit of the inhibitors into the mutant protein active sites. Each inhibitor was evaluated with its cognate wild-type protein from the crystal structure. (Analogous results were observed when a single protein was used.)
- For all six inhibitors the trend in surface area increase was the same: I84V generated the smallest increase, I84C generated a moderate increase, and I84A generated the largest increase. This trend in surface area increase is also evident in the trend in increase of unoccupied volume that is generated in each of these mutants (Illustrated with RTV, Figure 5). Decreased Van der Waal contacts with the inhibitors due to creation of unoccupied volume/increase in non-contact surface area is consistent with decreased inhibitor affinity for these mutants.
- Modeling of the p1/p7 and p1/p6 *gag* cleavage site mutants suggests that the mutant substrates containing valine and phenylalanine, respectively, fill the unoccupied volume created by the I84A mutation in the protease better than wild type alanine and leucine, respectively (Figures 6a and b).

Table 2. Surface Area Created by Residue 84 Mutations in HIV Protease

	Non-contact surface area created by suboptimal fit of inhibitor and mutation at 84					
	RTV	**SQV**	**NFV**	**LPV**	**APV**	**IDV**
I84V	65.5	36.7	36.0	20.6	33.2	42.5
I84C	108.8	62.3	112.8	30.0	56.3	96.1
I84A	193.0	147.5	150.8	174.2	158.5	171.1

All values in square angstroms (Å^2). Each inhibitor was evaluated within its cognate wild-type protein present in each crystal structure.

Figure 4. Crystal Structure of RTV Into the Active Site of WT HIV Protease, Showing the Surface That Contacts Ile 84; Both Symmetry-related Residues Are Shown

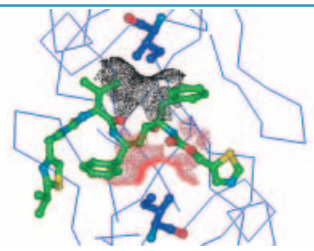
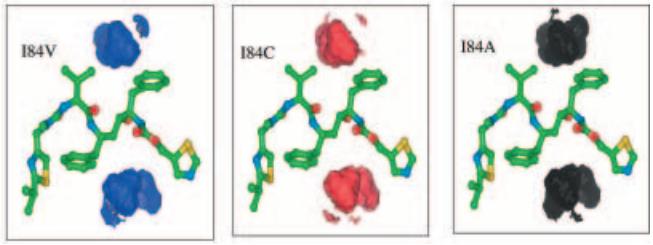


Figure 5. Volumes Created in Mutant Enzymes by the Suboptimal Fit of Inhibitors Within the Active Site



These three pictures show representative volumes created in the proteins (protein not shown) for the I84V (blue), I84C (red), and I84A (black) mutations, respectively. The inhibitor illustrated is ritonavir and is representative of the six inhibitors evaluated in this study. The size of these volumes are 55.0, 56.8, and 84.5 cubic Angstroms, respectively.

Figure 6a. Model of the mutant p7/p1 Gln-Val-Asn*Phe-Leu-Gly cleavage sequence within protease active site. The substrate P_2 Ala to Val mutation improves the hydrophobic fit within the larger cavity provided by the I84A mutation.

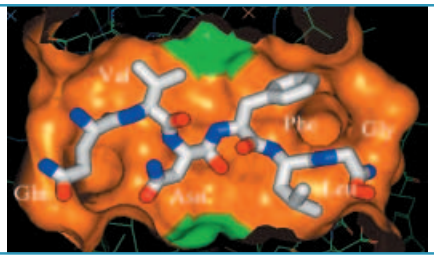
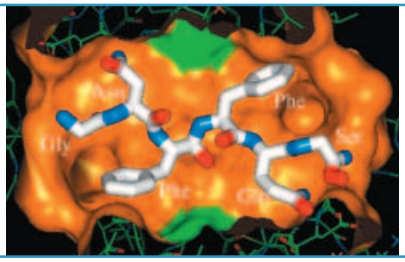


Figure 6b. Model of mutant p1/p6 substrate GNF*FQS in active site of I84A mutant protease. Surface of the Ala 84 residue (both symmetry-related residues) is shown in green. The location of the Leu-to-Phe mutation at the P1' position of the substrate is proximal to residue 84.



CONCLUSIONS

- A novel I84C protease mutation confers high-level resistance to NFV and SQV, intermediate resistance to RTV, IDV, and APV, and low-level resistance to LPV.
- The I84A mutation confers high-level of resistance to all PIs with the exception of a modest level of resistance to LPV.
- In the absence of I54V, isolates containing the I84V mutation display relatively modest changes in susceptibility to PIs.
- Increased resistance is accomplished by addition of other secondary mutations (common pathway) or by selection of I84C or I84A (less commonly).
- Replication capacity was impaired in both I84C and I84A mutants. The mutations in cleavage sites may play a role in partially restoring the RC of I84C and I84A mutants.
- Structural analysis of three-dimensional models of the mutations at position 84 and inhibitors or mutant gag substrates provide insight into the mechanisms by which these mutations contribute to resistance and RC.

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