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SCIENCE DIRECT
Virology 347 (2006) 405 – 409
VIROLOGY
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Co-evolution of nelfinavir-resistant HIV-1 protease and the p1–p6 substrate

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Received 9 September 2005; returned to author for revision 23 October 2005; accepted 29 November 2005
Available online 20 January 2006

Abstract

The selective pressure of the competitive protease inhibitors causes both HIV-1 protease and occasionally its substrates to evolve drug resistance. We hypothesize that this occurs particularly in substrates that protrude beyond the substrate envelope and contact residues that mutate in response to a particular protease inhibitor. To validate this hypothesis, we analyzed substrate and protease sequences for covariation. Using the χ^2 test, we show a positive correlation between the nelfinavir-resistant D30N/N88D protease mutations and mutations at the p1–p6 cleavage site as compared to the other cleavage sites. Both nelfinavir and the substrate p1–p6 protrude beyond the substrate envelope and contact residue 30, thus possibly making the p1–p6 cleavage site more vulnerable to co-evolution.

Keywords: HIV-1; Protease; Drug resistance; Co-evolution; p1–p6; D30N

HIV-1 protease inhibitors bind competitively to the active site of the enzyme (Wlodawer and Erickson, 1993). Mutations in the protease that alter inhibitor binding and cause drug resistance can also affect substrate recognition by changing the enzyme's substrate specificity. To compensate, the virus will be under selective pressure to co-evolve the substrate sequence, thereby allowing the protease to retain activity (Bally et al., 2000; Doyon et al., 1996; Mammano et al., 1998; Feher et al., 2002). Earlier studies from our laboratory have shown that substrate specificity of the protease is based on the shape adopted by the substrate sequences, defined as "the substrate envelope" (Prabu-Jeyabalan et al., 2002). Most primary active-site mutations occur outside the substrate envelope and thereby preferentially impact inhibitor binding over substrate recognition. Therefore, most of the substrates do not co-evolve with the protease. However, some substrates protrude beyond the envelope, and we observe that they are the ones which co-evolve with the protease.

In this study, we focus on the D30N and N88D protease mutations, which are a signature of nelfinavir (NFV) resistance (Pai et al., 1999; Patick et al., 1998) and their correlation with mutations at the p1–p6 substrate cleavage site. In the crystal structure of wild-type (WT) HIV-1 protease in complex with NFV, Asp30 forms a hydrogen bond with the m-phenol group of NFV (Kaldor et al., 1997), and superposition of NFV on the substrate envelope shows that this group protrudes from the envelope (Fig. 1a), suggesting that mutation at this residue will preferentially impact inhibitor binding over substrate recognition. The D30N mutation of aspartic acid to an asparagine in the protease likely results in a weaker hydrogen bond that destabilizes NFV binding. In this study, we present an analysis of Gag and Pol sequences from viral isolates of patients treated with protease inhibitors that reveals correlations between the D30N/N88D protease mutations and mutations within the corresponding p1–p6 cleavage site, which also makes direct ionic interactions with Asp30.

Sequence analysis

Viral sequences (N = 196) extending from Gag aa 346 to Pol aa 162 were obtained from patients who were part of the NARVAL trial (Meynard et al., 2002). These patients, treated with highly active antiretroviral therapy, had received a median of 3 prescribed protease inhibitors, for an average of 32

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doi:10.1016/j.virol.2005.11.049

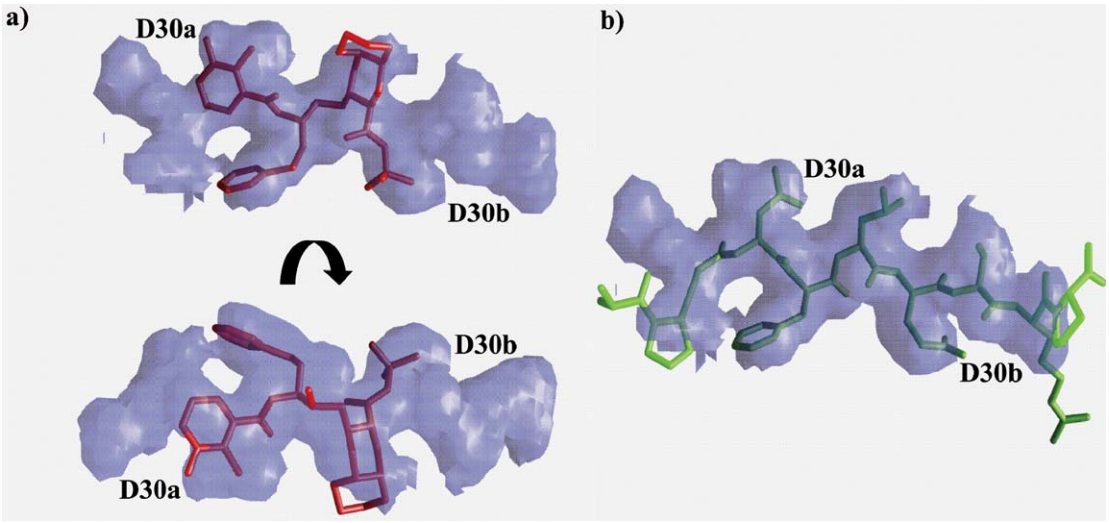

Fig. 1. NFV and p1–p6 both contact D30 outside the substrate envelope. (a) Shown in top and bottom views is NFV in red within the substrate envelope in blue. The m-phenol group of NFV protrudes to contact D30a outside the substrate envelope. (b) p1–p6 in green within the substrate envelope. GlnP2′ protrudes from the envelope and interacts with D30b.

months. Plasma viral RNA was extracted, and nested RT-PCR was performed to amplify the gag-protease region, including p2, p7, p1, p6 and the whole PR. Direct dideoxynucleoside terminator cycle sequencing of the PCR product was performed, and sequencing products were analyzed on an ABI 3100 (Perkin-Elmer ABI, Foster City, Calif., USA) instrument, and manually proof read and edited using Sequence Navigator software, in both 5′ and 3′ directions. Polymorphisms were defined as differences in amino acid usage with respect to the HIV-1 B subtype consensus sequence (Los Alamos HIV database, http://hiv-web.lanl.gov). When a mixture of wild-type and mutant residues was detected, the corresponding codon was classified as mutated. Over 90% of these isolates were subtype B. The viral sequences, which included the protease and 10 substrate cleavage sites, had a median of 6 protease mutations per sequence.

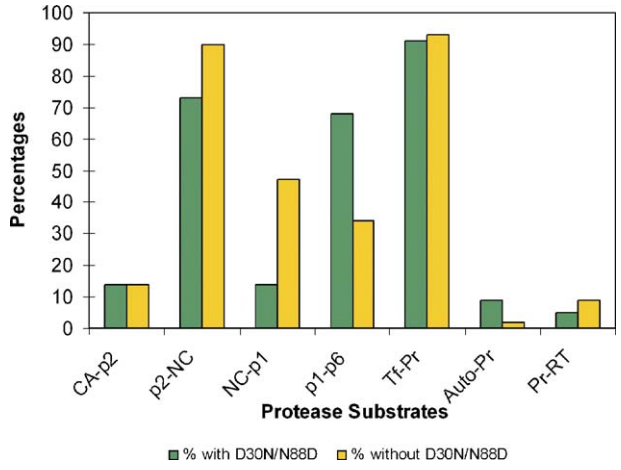

Fig. 2. Mutation rates at substrate cleavage sites with and without corresponding D30N/N88D protease mutations. Green bars indicate the simultaneous occurrence of cleavage-site mutations and the D30N/N88D protease mutations. Yellow bars represent percentages of isolates in which mutations occur in the cleavage sites in the absence of the D30N/N88D protease mutations.

The entire data set was read and entered into a Microsoft Access Database using software written in Visual Basic. Our analysis focused on qualitative changes in cleavage-site sequences, as compared to the HXB2 sequence, that covary with the D30N/N88D drug-resistant substitutions in the protease. For each protease sequence with the D30N/N88D substitutions in the enzyme, the corresponding substrate cleavage sites were scored for the presence or absence of mutations. Conversely, for every substrate site that scored positive for amino acid substitutions, changes at positions 30 and 88 in the enzyme were scored and tabulated.

The simultaneous occurrence of cleavage-site mutations and the D30N/N88D protease mutations were compared for each of the seven substrates studied (Fig. 2). The percentage of mutated substrate sequences that occurred with the D30N/N88D mutations in the corresponding protease sequence was calculated by dividing the number of mutated cleavage-site sequences by the total number of protease sequences with D30N/N88D mutations (N = 21). The percentage of mutated substrate sequences that did not occur with corresponding

Table 1
Covariation between substrate mutations and D30N/N88D protease mutations

Substrate cleavage site	Number of mutated cleavage-site sequences	χ^2	P value	Phi correlation coefficient
CA-p2	27	0.07	0.79	+0.01
p2-NC	175	2.13	0.14	−0.13
NC-p1	86	6.83	0.01	−0.2
p1–p6	74	10.18	0.001	+0.24
Tf-Pr	184	0.01	0.92	+0.03
Auto-Pr	5	0.16	0.16	+0.15
Pr-RT	16	0.03	0.86	−0.04

Total number of viral sequences isolated from patients = 196.
Total number of viral sequences with D30N/N88D protease mutations = 21.
Total number of viral sequences without D30N/N88D protease mutations = 175.