Effect of mutations on the dimer stability and the pH optimum of the human foamy virus protease

Tamás Sperka, Péter Boross, Helga Eizert, József Tózsér and Péter Bagossi

Introduction
The aspartyl protease (PR) of retroviruses plays a crucial role in the maturation of virus (Oroszlan and Luftig, 1990; Tózsér and Oroszlan, 2003) and may also have a role in the early phase of life cycle (Rumlova et al., 2003; Tózsér and Oroszlan, 2003). The protease of human immunodeficiency virus type 1 (HIV-1) is a good target for chemotherapy of AIDS, and protease inhibitors are now important components of the combination anti-retroviral therapy (Eron, 2000; Tózsér, 2003). Much less is known about the PR of another human retrovirus, the human foamy virus (HFV), which has several unusual features (Flügel and Pfrepper, 2003; Rethwilm, 2003), but it is a great promise for gene therapy (Mergia and Heinkelein, 2003). The HFV PR is essential for viral infectivity, since mutation of the active site Asp residues resulted in noninfectious virions (Konvalinka et al., 1995b), as previously found for HIV-1 PR (Kohl et al., 1988). HFV PR was expressed in a vaccinia virus system but it was not purified (Luukkonen et al., 1995). Later it was cloned and found to be active as part of a thioredoxin fusion protein, but its activity was lost after elimination of the heterologous protein part fused with the enzyme coding sequence (Pfrepper et al., 1997). HFV PR was also cloned with a C-terminal His-tag and this enzyme was used to determine the cleavage sites in the Gag and Pol polyproteins (Pfrepper et al., 1998, 1999) and to characterize the specificity of the enzyme (Pfrepper et al., 2001). We have cloned the HFV PR in fusion with maltose binding protein (MBP) and characterized the fusion protein (Fenyőfalvi et al., 1999). Recently the purification protocol was improved (Boross et al., 2006), and the modifications allowed us to prepare and characterize purified, processed and active HFV PR. Comparison of the processed and fusion forms of the wild-type and mutant (S25T) PRs suggested that the fusion forms can be used instead of the processed enzymes for comparative studies (Boross et al., 2006). The obtained catalytic constants for HFV PR were much lower than those we previously determined for various mammalian retroviral proteases coded on pol genes (Fenyőfalvi et al., 1999), but similar to those obtained previously with gag-encoded avian retrovirus PR (Tózsér et al., 1996). The pH optimum of HFV PR [6.6 (Fenyőfalvi et al., 1999)] was much higher than those values published for HIV-1 PR [4.1 (Hyland et al., 1991), 4.5–5.1 (Szeltner and Polgár, 1996b), 5.5 (Darke et al., 1989), depending on the used substrate, ionic strength and other experimental conditions]. Furthermore, the dimer stability of HFV PR was much lower than that of HIV-1 PR; half-maximal enzyme activity was reached at 0.75 M (Fenyőfalvi et al., 1999) as compared with 1.85 M (Wondrak et al., 1996) denaturant concentration, respectively. To explore the molecular basis of these unusual features, we have introduced several mutations near the catalytic aspartates.

Materials and methods
Site-directed mutagenesis of the HFV PR
Cloning of the wild-type and S25T mutant HFV PR in fusion with MBP were described previously (Fenyőfalvi et al., 1999; Boross et al., 2006). For single mutants, the wild-type residues were exchanged using the Quick-Change mutagenesis protocol (Stratagene, La Jolla, CA, USA) with the following oligonucleotide pairs obtained from Sigma-Genosys (The Woodlands, TX, USA). Mutated positions are indicated by underlined letters. Q8R: 5'-CTTCACTGCTTATGGCGCTTCGGCGG-G-3' and 5'-CGCCGGAAGCCGGGCTAACAAGCTAAG-3'; H22L: 5'-GGGACTAAATTGTTAGCC-3' and 5'-GGGACTAAATTGTTAGCC-3'; S25T: 5'-GTTGCCCTGAAATCCCAAGG-GCTAAACATTAGTCCC-3'; S25T: 5'-GTTAGCCCTCAGGATAGCCAGGGCCGAA-3' and 5'-GTTAGCCCTCAGGATAGCCAGGGCCGAA-3'; T28D: 5'-CCATGGGATTCCAGGGGACATACTGTTATCCCTG-GAAAG-3' and 5'-CTTCCAGAATCCAGCCATCTGTTATCCCTG-GAAAG-3'. For Q8R-T28D double mutant, the second mutation was introduced into the T28D mutant HFV PR using the above-mentioned Q8R oligonucleotide pairs.
For H22L-T28D double mutant, the second mutation was introduced into the T28D mutant HFV PR using the following oligonucleotides: 5'-GGGACATAATTTGGACCCCTCGG-GATTCCAGGGGACAGC-3' and 5'-GCTTGGCCCCTGTA-TCCAGAGGCTAACATTTAGTCCG-3'. Mutations were verified by DNA sequencing performed using the ABI Prism dye terminator cycle sequencing kit (Applied Biosystem, Foster City, CA, USA) and an ABI Model 373A sequencer (Applied Biosystem, Foster City, CA, USA).

**Purification of the wild-type and mutant HFV PRs**
A total of 500 ml freshly prepared Escherichia coli culture bearing the plasmid construct coding for the wild-type or a mutant enzyme was grown at 37°C up to an absorbance at 600 nm of 0.7–1.0, in Luria–Bertani medium containing 100 µg/ml ampicillin. Then induction with IPTG (1.0 mM) was performed for 5 h and cells were harvested by centrifugation at 2000 × g for 10 min at 4°C. After removal of the supernatant, 25 ml lysis buffer (50 mM Tris, pH 7.2, 1 mM EDTA and 100 mM NaCl) was added. Cells were disrupted by freezing–thawing followed by sonication on ice. Samples were centrifuged at 9000 × g for 15 min at 4°C. The supernatant was loaded on a column containing amylase resin (25 ml) applied to AKTApurifier automated liquid chromatography system (Amersham Pharmacia Biotech AB, Uppsala, Sweden) and extensively washed with the lysis buffer. The fusion protein was eluted with lysis buffer containing 20 mM maltose. The elution was followed at 280 nm. Fractions with high protein concentration were collected and the fusion protein was precipitated with ammonium-sulfate (4 M final concentration). The precipitate was dissolved in lysis buffer containing 0.1% β-mercaptoethanol. Purity of the enzyme preparations was assessed by SDS–PAGE, using 10–20% gradient gels, and protein concentrations were determined by the Bradford spectrophotometric method (Bradford, 1976). Rabbit anti-serum against the conserved active site region of the HFV protease (Morozov et al., 1997) was used for immunoblotting, performed according to Towbin et al. (1979). Rainbow molecular mass markers (Amersham Biosciences, Piscataway, NJ, USA) were used for comparison.

**Proteolytic assay**
Oligopeptide substrate SRAVN*TVTQS (where asterisk shows the site of cleavage) was synthesized as described previously (Fenyőfalvi et al., 1999). Kinetic parameters were determined in 50 mM MES, 100 mM Tris, 50 mM acetate, 1 M NaCl, pH 6.3 buffer (META). The pH of the buffer system was sensitive to the temperature, therefore it was adjusted at 37°C. The reaction mixtures contained 1.4–22 µM purified enzyme and 0.2–1.0 mM substrate and they were incubated at 37°C for 1 h. The reactions were stopped by the addition of 180 µl 1% trifluoroacetic acid, and an aliquot was analyzed by reversed-phase HPLC as described previously (Fenyőfalvi et al., 1999). Cleavage products of PR-catalyzed hydrolysis were previously identified by amino acid analysis for wild-type HFV protease (Fenyőfalvi et al., 1999) and mutant enzymes produced the same cleavage fragments as indicated by identical retention times. Kinetic parameters were determined by fitting the data obtained at <20% substrate hydrolysis to the Michaelis–Menten equation by using the Fig. P program (Fig. P Software Corp., Durham, NC, USA).

The pH optimum of the enzymes was determined in META buffer but having pH in the range of 3–9. Symmetrical bell-shaped pH optimum curves were fitted by nonlinear regression module of SigmaPlot program (Systat Software, Inc., Point Richmond, CA, USA) using the following equation (Polgár et al., 1994):

\[
\text{relative activity} = \frac{d_{\max}}{[1 + 10^{(pK_1-pH)} + 10^{(pH-pK_2)}]},
\]

where pK1 and pK2 are the negative logarithms of the acidic ionization constants of the catalytically competent ionizing groups.

The urea denaturation curves were determined in META buffer having pH 6.0 or 7.2 and in the presence of 0–5 M urea. Sigmoidal urea denaturation curves were fitted by the nonlinear regression module of SigmaPlot using the following equation (Jandu et al., 1990; Szeltner and Polgár 1996a):

\[
f_u = 1 - \exp \left( \frac{(m[D]-\Delta G^0)/(RT)}{1 + \exp \left( (m[D]-\Delta G^0)/(RT) \right)} \right),
\]

where \(f_u\) is fraction of unfolded protein, \([D]\) is the urea concentration, \(\Delta G^0\) is the conformational stability of the protein at zero concentration of urea, \(m\) is a measure of the dependence of \(\Delta G\) on the urea concentration, \(R\) is the gas constant and \(T\) is the absolute temperature. The urea concentration leading to 50% loss in enzymatic activity can be calculated from the following equation: \(D_{1/2} = \Delta G^0/m\).

**Sequence alignment and molecular modeling**
The sequences of the retroviral proteases were aligned to the sequences of structurally aligned HIV-1, HIV-2, SIV (simian immunodeficiency virus), EIAV (equine infectious anemia virus), FIV (feline immunodeficiency virus) and RSV (Rous sarcoma virus) proteases. Structural alignment was made by Whatif (Vriend, 1990), and the initial multiple sequence alignment was made by ClustalW (Thompson et al., 1994), followed by manual corrections based on the structural alignment. Homologous model of HFV PR was built by Modeller (Sali and Blundell, 1993) from the crystal structure of HIV-1 PR complexed with an inhibitor [PDB code: 7HVP (Swain et al., 1990)]. Structures were examined on Silicon Graphics workstations using Sybyl program package (Tripos, St Louis, MO, USA).

**Results and discussion**
HFV and its protease have several unusual features (Flügel and Pfrepper, 2003; Rethwilm, 2003). Our previous characterization of the HFV PR suggested that in comparison with HIV-1 PR the enzyme has lower catalytic efficiency, lower dimer stability and higher pH optimum (Fenyőfalvi et al., 1999; Boross et al., 2006). Therefore, various residues in the vicinity of the catalytic aspartates were selected for mutagenesis study (Figures 1 and 2) to explore their potential contributions to the unusual parameters. The activity of mutant MBP-HFV proteases was compared with that obtained for the wild-type fusion protein (Table 1). The specificity constant of S25T mutant was wild-type-like, but the values of T28D, Q8R and H22L single mutants were 2.6, 2.7 and 10.8 times lower than that of the wild-type protein, respectively. The specificity constant of H22L-T28D double mutant was also 4.2 times lower; however,
the corresponding value of Q8R-T28D double mutant was 3.2 times higher than that of wild-type fusion protein. This latter change was mainly caused by the decreased $k_{\text{cat}}$ value, which may suggest that complete regeneration of the Arg-Asp ion-pair may stabilize the enzyme–substrate complex. Most of the mutants showed $k_{\text{cat}}$ values close to the wild-type value, implying that the folding capability of these mutants is likely similar to that of the wild-type. Unfortunately, there is no tight-binding inhibitor for HFV PR suitable for active site titration; therefore, enzyme concentrations were determined by protein concentration measurements. These enzyme concentrations were used for calculation of the apparent $k_{\text{cat}}$ values from the experimentally measured $V_{\text{max}}$ values.

The small variation of the apparent $k_{\text{cat}}$ values of the mutant enzymes compared with that of the wild-type enzyme may reflect small variation of the ‘true’ catalytic constant and in parallel small variation in the concentration of the active, correctly folded enzyme. The other possibility to get unchanged $V_{\text{max}}$ value requires an increased ‘true’ catalytic constant and a decreased folding capability or vice versa, which is highly unlikely.

We have determined the pH profile of the wild-type and the mutant fusion enzymes (Table II). The lowest pH optimum was about 6.8 for the T28D mutant. Dimer stabilities of the wild-type and mutant fusion enzymes have been compared by measuring their urea denaturation curves at two pH values: 6.0 and 7.2 (Table III). These values were located at the two sides of the bell-shaped curves on the activity versus pH plots.

Sequence alignment of retroviral proteases (Figure 1) showed that several unusual residues can be found around the active site triplet: the residue corresponding to His22 is Leu in all other retroviral PRs (Leu23 in HIV-1 PR) except those of foamy viruses in which aromatic residue (Phe or Tyr) also can be found. All retroviral proteases contain a hydrophobic patch around the catalytic aspartates that may help to isolate the catalytic residues from the aqueous environment for maintaining the proper catalytic power and it also may contribute to the interaction energy of the dimer by providing hydrophobic contacts between the monomers. A hydrophobic cluster is formed by Leu10, Leu23, Ala28, Val82 and Ile84 residues which may suggest that complete regeneration of the Arg-Asp interaction energy of the dimer by providing hydrophobic contacts between the monomers. A hydrophobic cluster is formed by Leu10, Leu23, Ala28, Val82 and Ile84 residues which may suggest that complete regeneration of the Arg-Asp interactions between the monomers. A hydrophobic cluster is formed by Leu10, Leu23, Ala28, Val82 and Ile84 residues which may suggest that complete regeneration of the Arg-Asp interactions between the monomers.

An important feature of the aspartyl proteases is the -Asp-Thr/Ser-Gly- triplet at the active site (Figure 4), which forms the ‘fireman’s grip’ between the two subunits of the homodimeric retroviral proteases, or between the two domains of the single-chain cellular aspartyl proteases. The HFV PR contains a Ser at the active site triplet instead of Thr may form less stable dimers (Bagossi et al., 1996). A detailed study on the role of Ser in the protease of HIV-1 PR demonstrated that it is crucial for stabilization of the PR dimer and for overall stability of the protease.

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### Table I: Sequence comparison of N-terminal parts of retrovirus proteases

<table>
<thead>
<tr>
<th>Sequence alignment of retrovirus proteases</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
</tr>
<tr>
<td>HIV-1</td>
</tr>
<tr>
<td>HIV-2</td>
</tr>
<tr>
<td>HIV-3</td>
</tr>
<tr>
<td>HIV-4</td>
</tr>
<tr>
<td>HIV-5</td>
</tr>
</tbody>
</table>

### Table II: Urea denaturation curves of wild-type and mutant fusion enzymes

<table>
<thead>
<tr>
<th>pH</th>
<th>Urea concentration (M)</th>
</tr>
</thead>
<tbody>
<tr>
<td>6.0</td>
<td>0.0</td>
</tr>
<tr>
<td>7.2</td>
<td>0.0</td>
</tr>
</tbody>
</table>

### Table III: Dimer stabilities of wild-type and mutant fusion enzymes

<table>
<thead>
<tr>
<th>pH</th>
<th>Dimer stability (kcal/mol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>6.0</td>
<td>0.0</td>
</tr>
<tr>
<td>7.2</td>
<td>0.0</td>
</tr>
</tbody>
</table>

**Fig. 1.** Sequence comparison of N-terminal parts of retrovirus proteases. Manual alignment is given, based on sequence and structure comparison made with ClustalW and Whatif programs, respectively. The active site aspartates are shown in bold. Foamy virus proteases are shown in italics. Mutated positions are indicated by gray boxes.
the enzyme, but it is not absolutely required for activity (Strisovsky et al., 2000; Ingr et al., 2003). It was also shown that dimer stabilities of the Thr-containing HIV-1 and myeloblastosis-associated virus proteases were about one order of magnitude higher than the corresponding Ser-containing forms (Ingr et al., 2003). Our recent study showed that the specificity constant of HFV PR did not alter when Ser25 was changed to Thr, hence the presence of Ser in the active site triplet does not appear to be an important determinant for the low catalytic efficiency of the enzyme (Boross et al., 2006). However, the dimer stability was increased by the Ser25 to Thr mutation (Table III), in good agreement with the role of this residue in dimerization. It is of interest to note that mutation of Ser to Thr in the active site triplet increased the pH optimum. Aspartyl proteases are usually active at acidic pH, since the catalytically competent enzyme has one protonated and one deprotonated Asp at the active site, and this can be typically achieved at an acidic pH. However, exceptions are also known, for example renin is active in the physiological pH (Inagami et al., 1984). The increased pH optimum might be a consequence of increased dimer stability. The dimerization of HIV-1 PR is strongly dependent on the pH, forming less stable dimers at higher pH (Darke et al., 1994; Szeltner and Polgár, 1996a). The reverse mutation in HIV-1 PR (T26S) resulted in a lower pH optimum, as compared with that of the wild-type enzyme (Konvalinka et al., 1995a; Strisovsky et al., 2000), and the T26S mutant retained substantially smaller activity at pH 6.8 than the wild-type enzyme, in comparison with their activity at pH 4.7 (Strisovsky et al., 2000). While the pH optimum of Ser-containing wild-type HFV PR was also lower than the pH optimum of Thr-containing S25T mutant, the urea stability of S25T mutant increased at higher pH.

The conserved Arg8-Asp29 (Figure 5) and Arg8'-Asp29 ion-pairs at the outer side of the substrate-binding site in HIV-1 PR significantly contribute to the stability of the dimer (Lapatto et al., 1989; Loeb et al., 1989; Miller et al., 1989; Wlodawer et al., 1989; Wlodawer and Erickson, 1993; Manchester et al., 1994). However the corresponding residues in HFV PR are Gln8 and Thr28, respectively, which cannot form an ion-pair. To study the role of these residues in stabilization of the enzyme structure, two single mutants as well as

### Table I. Kinetic parameters determined for the wild-type and mutant HFV proteases in fusion with MBP for substrate SRAVN*TVTQS

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>(K_m) (mM)</th>
<th>(k_{cat}) (s(^{-1}))</th>
<th>(k_{cat}/K_m) (mM(^{-1}) s(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wild-type</td>
<td>0.41 ± 0.02</td>
<td>0.0053 ± 0.0001</td>
<td>0.013 ± 0.001</td>
</tr>
<tr>
<td>Q8R</td>
<td>1.3 ± 0.3</td>
<td>0.0061 ± 0.0008</td>
<td>0.0047 ± 0.0012</td>
</tr>
<tr>
<td>H22L</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>S25T</td>
<td>0.26 ± 0.04</td>
<td>0.0031 ± 0.0001</td>
<td>0.012 ± 0.002</td>
</tr>
<tr>
<td>T28D</td>
<td>0.35 ± 0.06</td>
<td>0.0017 ± 0.0001</td>
<td>0.0049 ± 0.0009</td>
</tr>
<tr>
<td>Q8R-T28D</td>
<td>0.17 ± 0.02</td>
<td>0.0071 ± 0.0002</td>
<td>0.042 ± 0.005</td>
</tr>
<tr>
<td>H22L-T28D</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

*Due to the apparently high \(K_m\) values for these enzymes the \(k_{cat}/K_m\) value was calculated from measurements performed in first-order kinetic conditions.

### Table II. \(pK_1\), \(pK_2\) and pH optimum values for wild-type and mutant HFV PRs determined for MBP fusion proteins using substrate SRAVN*TVTQS

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>(pK_1) (±SE)</th>
<th>(pK_2) (±SE)</th>
<th>pH optimum (±SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wild-type</td>
<td>5.61 ± 0.16</td>
<td>7.00 ± 0.15</td>
<td>6.30 ± 0.11</td>
</tr>
<tr>
<td>Q8R</td>
<td>5.71 ± 0.26</td>
<td>6.70 ± 0.24</td>
<td>6.21 ± 0.18</td>
</tr>
<tr>
<td>H22L</td>
<td>5.64 ± 0.24</td>
<td>6.83 ± 0.23</td>
<td>6.24 ± 0.17</td>
</tr>
<tr>
<td>S25T</td>
<td>5.49 ± 0.15</td>
<td>7.81 ± 0.18</td>
<td>6.65 ± 0.12</td>
</tr>
<tr>
<td>T28D</td>
<td>5.79 ± 0.14</td>
<td>7.82 ± 0.16</td>
<td>6.81 ± 0.11</td>
</tr>
<tr>
<td>Q8R-T28D</td>
<td>5.59 ± 0.15</td>
<td>7.58 ± 0.17</td>
<td>6.59 ± 0.11</td>
</tr>
<tr>
<td>H22L-T28D</td>
<td>6.01 ± 0.29</td>
<td>6.98 ± 0.29</td>
<td>6.50 ± 0.20</td>
</tr>
</tbody>
</table>

*The pH optimum of HIV-1 PR was 4.1 (Hyland et al., 1991), 4.5–5.1 (Szeltner and Polgár, 1996b), 5.5 (Darke et al., 1989), depending on the used substrate, ionic strength and other experimental conditions.
Q8R-T28D double mutant were constructed. The T28D and the Q8R single mutants showed higher stability against urea than the wild-type enzyme (Table III). We expected only marginal effect in the case of single mutants, when only the half of the ion-pair was regenerated, and a more pronounced effect was expected in the case of the double mutant. However, the T28D mutant was even more stable than the double mutant Q8R-T28D enzyme, which suggests that other structural features may also play a role. The stability values of these enzymes were separated into two groups: low stability group consisted of the Q8R mutant and the wild-type enzymes, while T28D and Q8R-T28D mutants had higher $D_{1/2}$ values at both pH values. The stability of enzymes correlated with the hydrogen-bond forming capability of these residue pairs. Only one hydrogen bond can be formed between Gln-Thr and Arg-Thr residue pairs in contrast to Gln-Asp and Arg-Asp pairs, where two hydrogen bonds can be formed. It is also possible that the HFV PR structure significantly deviates from the known retroviral protease fold, which may cause, at least partially, the unusual features of the HFV PR. Recently, the crystal structure of human T-cell leukemia virus type 1 (HTLV-1) protease was solved and showed an example for unexpected structural variations (Li et al., 2005). However, specific structural features of HFV PR remain unpredictable until an experimental structure of a protease belonging to foamy virus family will be solved. Interestingly, T28D mutant has the highest pH optimum among the studied mutant forms of HFV PR (Table II).

Our results showed that several features of HFV PR were substantially different from those of other retroviral proteases. The pH optimum of wild-type HFV PR was higher than that of HIV-1 PR, but the wild-type sequence was not optimized for that: all mutants had the same or higher pH optimum than the wild-type HFV PR. Interestingly, the studied mutants showed the same or higher stability against urea at both pH values, suggesting that during evolution HFV PR did not evolve to maximize the dimerization energy, as compared with HIV-1 PR.

![Table III. Stability parameters for wild-type and mutant HFV PRs determined using MBP fusion proteins](image_url)

**Table III.** Stability parameters for wild-type and mutant HFV PRs determined using MBP fusion proteins

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>pH = 6.0</th>
<th>pH = 7.2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$\Delta G^0 \pm SE$ (kJ/mol)</td>
<td>$m \pm SE$ (kJ/mol/M)</td>
</tr>
<tr>
<td>Wild-type</td>
<td>-6.48 ± 0.66</td>
<td>-11.88 ± 1.10</td>
</tr>
<tr>
<td>Q8R</td>
<td>-5.82 ± 0.85</td>
<td>-9.60 ± 1.25</td>
</tr>
<tr>
<td>H22L</td>
<td>-5.33 ± 0.80</td>
<td>-7.76 ± 1.02</td>
</tr>
<tr>
<td>S25T</td>
<td>-8.31 ± 0.80</td>
<td>-10.00 ± 0.92</td>
</tr>
<tr>
<td>T28D</td>
<td>-6.74 ± 0.64</td>
<td>-7.86 ± 0.70</td>
</tr>
<tr>
<td>Q8R-T28D</td>
<td>-7.47 ± 1.14</td>
<td>-9.31 ± 1.33</td>
</tr>
<tr>
<td>H22L-T28D</td>
<td>-5.50 ± 0.76</td>
<td>-7.38 ± 0.91</td>
</tr>
</tbody>
</table>

$^a$ $D_{1/2}$ value for HIV-1 PR: 1.85 M (Wondrak et al., 1996).

![Fig. 3. Hydrophobic residues being around the catalytic aspartates in the crystal structure of HIV-1 PR.](image_url)
While the wild-type HFV PR had the same sensitivity against urea at both pH values, mutant enzymes showed higher sensitivity against urea at pH 6.0 than at pH 7.2. The overall results of these mutational studies suggest that requirements of HFV PR structure may differ from that of other retroviral protease structures, in response to a different selective pressure caused by the different life cycle of foamy viruses. Unlike conventional retroviruses, the majority of mature, enveloped, infectious foamy virus particles remain in the endoplasmic reticulum and only ~5% can be found in the cell culture supernatant (Yu and Linial, 1993; Linial and Eastman, 2003). Since it was shown that the ProPol polyprotein is not efficiently cleaved between PR and RT (Flügel and Pfrepper, 2003), it is also possible that C-terminal flanking sequences, including the reverse transcriptase of Pol polyprotein, may modify the features of HFV PR, for example by providing additional dimerization interfaces, which are not provided after maturation of other viral proteases.

Acknowledgements

The authors thank Gábor Bakondi for the help in protein purification and protease assays and Szilvia Pető for the technical assistance. This research was sponsored by the Hungarian Science and Research Fund (OTKA F34479, F35191, T43482) and the Ministry of Public Health and Welfare (ETT 88/2003).

Fig. 4. Hydrogen bonds around the catalytic aspartates (‘fireman’s grip’) in the crystal structure of HIV-1 PR. Arrows indicate the possible hydrogen bonds, and the distances in Angstrom units are also provided. Residues of the second monomer are labeled by a primed number.

Fig. 5. Ion-pair of Arg8 and Asp29' in the crystal structure of HIV-1 PR. Arrows indicate the strong ionic/hydrogen-bond interaction, with distances in Angstrom units. Residue of the second monomer is labeled by a primed number.

References


Received October 10, 2005; revised May 17, 2006; accepted May 19, 2006

Edited by Angelo Fontana