Comparison of the Crystal Structures and Intersubunit Interactions of Human Immunodeficiency and Rous Sarcoma Virus Proteases*

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The crystal structures of the proteases (PRs) encoded by the Rous sarcoma virus (RSV) and the human immunodeficiency virus (HIV) have been compared. The crystallographic monomer of HIV PR superimposes on the two crystallographically independent subunits of the RSV PR dimer with root mean square deviations of 1.45 and 1.55 Å for 86 and 88 common Ca atoms, respectively. There is a conserved structural core consisting of seven β-strands forming two perpendicular layers, a β-helix, and the amino- and carboxyl-terminal β-strands. PRs from related retroviruses fold into similar structures with surface turns of variable length between the β-strands. Both HIV and RSV PR dimers have significant subunit-subunit interactions in three regions: the "firemen's grip" at the active site; the salt bridges involving Arg$_6$, Asp$_{28}$, and Arg$_{67}$ of HIV PR; and the termini of the two subunits, which form a four-stranded antiparallel β-sheet. The specific interactions of the termini differ in the two PRs. The carboxyl termini, residues 96–99 of HIV PR and residues 119–124 of RSV PR, contribute ~50% of the intersubunit ionic and hydrogen bond interactions and ~45% of the buried surface area involved in dimer formation. This information may be useful in the design of site-directed mutations or inhibitors of dimer formation.

EXPERIMENTAL PROCEDURES

The coordinates for HIV PR correspond to Set 3HVP, and the RSV PR coordinates are Set 2RSP in the Brookhaven Protein Data Bank. The individual subunits were superimposed using Ca atoms with the program ALIGN (Satow et al., 1984) and were analyzed with the program DISTRMS, which calculated root mean square deviations for Ca, main chain, and side chain atoms and all common atoms of residues 35–57. The structures were examined on an Evans & Sutherland PS390 computer graphics system using FRODO (Jones, 1971). A probe size of 1.4 Å was used.

RESULTS AND DISCUSSION

HIV-1 PR crystallizes as a dimer of two identical subunits (imposed by crystal symmetry), whereas the RSV PR dimer contains two crystallographically independent subunits. The HIV PR subunit superimposes with root mean square deviations of 1.55 Å for 88 Ca atoms and 1.45 Å for 86 common Ca atoms on the two nearly identical subunits of RSV PR (Fig. 1, upper). The overall topology is very similar, although HIV-1 PR of 99 residues is considerably shorter than 124-residue RSV PR. All of the deletions in HIV PR relative to RSV PR occur at surface loops. The two largest deletions are between β-strands b and c, and b' and c'. The region leading up to the flap has a different conformation, with a root mean square deviation of over 2.3 Å for all atoms of residues 35–57. The helical turn between β-strands d and a' in RSV PR is absent in HIV-1 PR. Residues 61–70 are not visible in the RSV PR electron density maps (Miller et al., 1989a), and the flap in HIV-1 PR is involved in crystallographic contacts with a symmetry related flap and other residues; and comparison is not straightforward. The single flap of nonviral aspartic proteases has a different conformation (Blundell et al., 1985; Suguna et al., 1987).
Comparison of HIV and RSV PR Crystal Structures

Fig. 1. Upper, stereo view of Ca chain of one subunit of HIV PR (continuous lines) superimposed on B subunit of RSV PR (dashed lines). Every 10th residue of RSV PR is labeled. The flap from residues 61 to 69 is not visible in RSV PR. Lower, modification of drawing by Jane Richardson (Duke University) of RSV PR dimer. The subunit on the left shows the nomenclature for the secondary structure, with β-strands a-d and a'-d' and helix h' (Blundell et al., 1985). β-Strands b, c, b', and c' form the A layer, and β-strands c, d, c', and d' form the B layer (Pechik et al., 1989). The amino and carboxyl termini are indicated (N and C, respectively). The corresponding sequence alignment is given by Weber (1989). The subunit on the right is shaded where the structure of HIV PR differs from that of RSV PR, usually due to shorter surface turns. The active site is indicated by an arrow, and the dashed lines represent the residues of the flap which were not visible in the electron density map for RSV PR.

All of the known retroviral PR sequences can be fit into a similar alignment, which has a conserved structural core and surface loops of varying lengths (Weber, 1989). HIV PR has the smallest and most compact structure. The conserved core structure consists of the A layer (β-strands b, c, b' and c'), which is almost perpendicular to the B layer (β-strands c, d, c' and d') (Pechik et al., 1989), the carboxyl-terminal helix, the flexible flap, and β-strands a and q at the termini (Fig. 1, lower). The 79 residues forming the core structure have 31% sequence identity and 1.43- and 1.57-Å root mean square deviations between main chain atoms of HIV compared to the two independent subunits of RSV PR (excluding residues 36-55 in the flap of HIV PR). This is comparable with differences observed between other pairs of protein structures with ∼30% sequence identity (Chothia and Lesk, 1986).

Dimer Interactions—In retroviruses, PR is produced as part
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Fig. 2. Stereo view of superimposed active site residues of two PRs. Asp$^{15}$, Thr$^{26}$, Gly$^{27}$, and Ala$^{28}$ from both subunits of the HIV PR dimer are shown by thick lines, and the corresponding residues 37-40 of the RSV PR dimer are shown by thin lines. The hydrogen bond interactions forming the fireman's grip are indicated by dashed lines.

Fig. 3. Stereo view showing HIV PR residues 28-31, 87, and 88 and 5' to 8' with interactions involving Arg$^{86}$, Asp$^{87}$, Asp$^{88}$, and Thr$^{111}$ (upper) and RSV PR residues 40-43, 111, and 112 and 5' to 10' with interactions involving Arg$^{100}$, Asp$^{111}$, Asp$^{112}$, and Thr$^{115}$ (lower). A prime indicates the second subunit in the dimer. Dashed lines indicate hydrogen bond or ionic interactions.
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**FIG. 4. Comparison of dimer interactions in four-stranded \( \beta \)-sheet formed by amino and carboxyl terminal.**

Upper, HIV-1 PR residues 1-4 and 96-99 from both subunits; lower, RSV PR residues 1-5 and 119-124 from both subunits. A prime indicates the second subunit of the dimer. Only the side chains involved in interactions are shown. Hydrogen bond interactions are indicated by **dashed lines**, and **positive and negative charges** indicate ionic interactions. Additional interactions with nearby residues are shown.

of a long polypeptide and is released by autocatalytic cleavage (Kramer et al., 1988). Since retroviral PRs are active as dimers, one assumption is that two polypeptides must be correctly aligned to form a PR dimer before autocatalysis may occur. It has been suggested that inhibiting formation of the PR dimer may provide a useful treatment for diseases caused by retroviruses (Wlodawer et al., 1989). Therefore, the inter-subunit interactions of RSV and HIV PRs have been described to understand the molecular basis for dimer formation. Intersubunit interactions occur in several regions: at the active site, between residues from helix h' and \( \beta \)-strands b and d, between the ends of the flaps, and between amino- and carboxyl-terminal \( \beta \)-strands a and q (Fig. 1, lower). In HIV PR, NH and C=O of Gly\( ^{19} \) at the end of the flap form hydrogen bonds to C=O and NH of Gly\( ^{22} \) in the flap of the other subunit. No comparison can be made with RSV PR since the equivalent residues of the flaps are apparently disordered.

The active site of the PR dimer includes residues from both subunits. The triplet Asp-Ser/Thr-Gly is followed by Ala in retroviral PRs or by Ser or Thr in almost all nonviral aspartic proteases. These 4 residues interact with the same 4 residues from the other subunit of the PR dimer in an arrangement which has been named the "fireman’s grip" (Blundell et al., 1985). The active sites of the two retroviral PRs superimpose with a 0.49-A root mean square deviation for all 44 atoms in the 8 residues (Fig. 2). Nonviral aspartic proteases differ only in the additional interaction formed by Ser or Thr following the conserved triplet (James and Sielecki, 1983; Miller et al., 1989a). The effect of Ser or Thr instead of Ala\( ^{40} \) in RSV PR has been tested by site-directed mutagenesis (Leis et al., 1990). Since the catalytic residues of all aspartic proteases are remarkably similar, this region may not provide a good target for a selective inhibitor of HIV-1 PR.

Asp\( ^{20} \) forms salt bridges with Arg\( ^{97} \) from helix h' and with Arg\( ^{97} \) at the end of \( \beta \)-strand b', and the side chains of Thr\( ^{3'} \) in \( \beta \)-strand d and of Asn\( ^{88} \) in the helix form a hydrogen bond interaction (Fig. 3, upper). A similar network of interactions between equivalent residues is observed in RSV PR (Fig. 3, lower), although Arg\( ^{97} \) is next to a deletion in HIV PR relative to RSV PR. Mutations of these residues in HIV PR reduce or eliminate protease activity (Louie et al., 1989; Loeb et al., 1989). These include the conservative substitutions of Lys for Arg\( ^{97} \) or Glu for Asp\( ^{20} \), which is consistent with a conserved conformation. Although Thr\( ^{3'} \), Arg\( ^{97} \), and Asp/Asn\( ^{88} \) are highly conserved in all PRs, the residue corresponding to Asp\( ^{20} \) in HIV PR is Glu, and the residue corresponding to Arg\( ^{97} \) is Glu in Moloney murine leukemia virus and feline leukemia virus (Weber, 1989). The conformation would be conserved if the same residues formed a different set of interactions in which Arg at position 87 formed a salt bridge with Glu at position 29 and a hydrogen bond with Gin 29. This could be tested by making multiple substitutions of these residues which were designed to form alternative interactions.

The amino and carboxyl termini of the two PRs form a four-stranded antiparallel \( \beta \)-sheet, in which the amino terminus of one subunit lies next to the carboxyl terminus of the adjacent subunit (Fig. 4). This arrangement is distinctly different from the interdomain six-stranded \( \beta \)-sheet seen in the pepsin-like proteases (Blundell and Pearl, 1989). In addition to the hydrogen bonds between main chain C=O and NH groups, there are further interactions which differ in the two PR dimers. In HIV PR, there is a salt bridge between the amino terminus of one subunit and the carboxyl terminus of the other subunit. The carboxyl terminus can also form an intersubunit hydrogen bond interaction with His\( ^{89} \) in \( \beta \)-strand c'. There is a network of hydrogen bonds linking the side chains of Asn\( ^{88} \) and Gin\( ^{29} \) from both subunits. Deletion of Phe\( ^{89} \) inactivates HIV PR (Hostomsky et al., 1989), although several amino acid substitutions can be accommodated (Loeb et al., 1989), which suggests that the length of the carboxyl terminus is important.
In RSV PR, there is no salt bridge between the termini. Instead, the carboxyl terminus forms a salt bridge with Arg219 from the other subunit in the dimer (Fig. 4, lower). The amino terminus forms a network of interactions involving Glu26, Asn199, and Asn248. Leu234 at the carboxyl terminus is dislocated out of the β-sheet on the surface of the protein. Other PRs, such as Moloney murine leukemia virus PR, have additional carboxyl-terminal residues which may be accommodated by a continuation from the position of Leu24 in RSV PR.

The terminal β-sheet has hydrophobic side chains facing the interior of the protein close to the active site. Leu97 is adjacent to Leu24 and Thr25 in HIV PR, whereas Leu210 is near Ser26 of the catalytic tripeptide in RSV PR. In other retroviruses, Leu97 is replaced by Ile and Leu24 by Val, which suggests that the internal hydrophobic packing is conserved (Weber, 1989). Mutation of Leu24, Thr25, or Leu97 reduces the HIV PR activity (Loeb et al., 1989) so that altering the internal packing may perturb the catalytic activity.

In HIV PR, a total of 34 hydrogen bond and four ionic interactions occur between the two subunits: two hydrogen bonds occur between the flaps; five occur between active site residues 6–8, 29, and 87 (Fig. 3); and 19 hydrogen bonds and two ion pairs are formed by terminal residues 1–4 and 96–99 (Fig. 4, upper). In fact, residues 96–99 contribute 50% of the ionic and 56% of the hydrogen bond intersubunit interactions. Similarly, the subunit-interactions of RSV PR involve 35 hydrogen bond and three ionic interactions: six hydrogen bonds occur between active site residues 37, 39; 11 occur between residues 6–10, 41, 111, and 115; and 18 hydrogen bond and two ionic interactions are formed by terminal residues 1–5 and 119–124. Again, the carboxyl termini contribute most of the intersubunit interactions. In the case of the RSV PR dimer, these calculations are incomplete since ~10 residues of each flap are not visible in the electron density maps (Jaskolski et al., 1990). Calculation of solvent-accessible surface area (Table I) shows that ~3000 Å² of surface area is buried on dimer formation in both RSV and HIV PRs. This is an estimate of the hydroporphic contribution to dimer formation. Carboxyl-terminal residues 96–99 of HIV PR or residues 120–124 of RSV PR contribute at least 44% of the buried surface area. Since the two carboxyl termini also contribute at least 50% of the ionic and hydrogen bond intersubunit interactions, these residues are expected to have a significant effect on dimer formation. This suggests that using peptides as competitive inhibitors of β-sheet formation by the amino and carboxyl termini may efficiently inhibit dimer formation and thus reduce PR activity. Interference with subunit-subunit interactions at the carboxyl terminus is the presumed mechanism for the specific inhibition of herpesvirus ribonucleotide reductase by peptides corresponding to the carboxyl terminus of the small subunit (Dutia et al., 1986; Cohen et al., 1986).

The prediction is that a peptide containing residues 96–99 of HIV PR or residues 120–124 of RSV PR should interfere with formation of the PR dimer. Unlike active site inhibitors, this type of inhibitor is likely to be specific for retroviral, rather than nonviral, aspartic proteases. The large contribution of the carboxyl termini to the intersubunit hydrogen bond and ionic interactions, which differ in RSV and HIV PRs, suggests that it may be possible to design an inhibitor of dimer formation that will be specific for one particular retrovirus, for example, HIV.

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Table I

| Subunit 1 | Subunit 2 | Dimer | Decrease on dimer formation | Contribution of C-termini |
|----------|----------|-------|-----------------------------|--------------------------|
| A²       | A²       |       | A²                          | A² (%)                   |
| HIV PR   | 6,659.1  | 6,659.1 | 10,094.5                    | 2,323.7                  |
| HIV PR without 96–99 | 6,189.5  | 6,189.5  | 10,591.1                    | 1,787.9                  |
| RSV PR   | 7,492.9  | 7,589.6  | 12,131.2                    | 2,951.3                  |
| RSV PR without 110–124 | 6,842.4  | 6,064.4  | 12,266.6                    | 1,641.2                  |

* This does not include residues 61–70 of the flaps.
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