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Genetics

# Human immunodeficiency virus proteinase dimer as component of the viral polyprotein prevents particle assembly and viral infectivity

**(polyprotein processing/AIDS/dimerization)**

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ABSTRACT **Enzymatically active retroviral proteinases are dimers of identical polypeptide chains with a fold similar to that of other aspartic proteinases. Each polypeptide chain, encoded on one of the viral polyproteins, is less than half the size of cellular aspartic proteinases and contains only one of the two active-site aspartate residues. A plasmid was constructed to generate a genetically linked dimer of the proteinase (PR) of human immunodeficiency virus (HIV) type 1, composed of two copies of the PR sequence linked by a structurally flexible hinge region. The expression product was stable and active against HIV polyprotein substrates. Mutational analysis revealed that the linked dimer, and not multimers thereof, contained the proteolytic activity. Expression of the linked dimer as a component of a HIV polyprotein by *in vitro* translation gave rapid autocatalytic processing, whereas the wild-type polyprotein was stable on prolonged incubation. Transfection of HIV subviral or proviral constructs, containing the linked dimer of HIV PR, gave premature processing of the viral polyproteins, thus preventing particle formation and infectivity. Premature processing also led to increased cell toxicity.**

The components of the retroviral nucleocapsid are synthesized as polyprotein precursors (*gag* and *gag-pol*) that are cleaved to the mature products by the viral proteinase (PR; for nomenclature of retroviral proteins; see ref. 1), which is itself part of the gag-pol polyprotein (2). Proteolytic processing is not required for particle formation but it is necessary to render these particles infectious (3). Retroviral polyproteins, in contrast to, e.g., picornaviral polyproteins, are not processed during or immediately after translation, but intact polyproteins are transported to the site of virus assembly and processing takes place only during and after budding of viral particles from the plasma membrane (reviewed in ref. 2). This mechanism of synthesizing different stable polyproteins at defined rates enables the virus to target many components of the particle to the site of assembly using only a single targeting signal. It requires, however, that an inactive form of a highly specific PR is targeted to the assembly site where it is activated only upon budding of the particle.

Retroviral PRs have been assigned to the aspartic PRs on the basis of sequence homology (4), biochemical and mutational analysis (ref. 5; reviewed in ref. 6), and crystallographic determination of the three-dimensional structure (7). Cellular aspartic PRs have two homologous domains contributing one aspartate residue of the active site each, whereas the viral PRs are less than half as long [99 amino acids in the case of human immunodeficiency virus (HIV)] and could correspond to only one such domain. It was therefore proposed that the HIV PR is a dimer of identical PR polypeptide chains, with a structure similar to other aspartic PRs (8). This was subsequently confirmed by structural analysis (7). Synthesis of a monomeric form of the retroviral PR may either indicate a regulatory role for PR dimerization in viral assembly and maturation or only reflect the need to reduce the genome size in view of the high error frequency of reverse transcription.

Crystallographic analysis of HIV PR (7) revealed that the subunits of the dimer are related by a nearly perfect twofold axis of symmetry. The terminal strands of two monomers form a common four-stranded antiparallel $\beta$-sheet (the dimer interphase) where the two C-terminal strands are flanked by the two N-terminal strands. These observations imply that the C-terminal end of the first molecule is located very close to the N terminus of the second molecule, suggesting that it should be possible to covalently link the two monomers by a short hinge region without distorting the overall structure of the enzyme. In this report, I describe the construction of vectors for expression of a single-chain dimer of HIV PR and the effects of this covalently linked dimer on polyprotein processing, virus assembly, and infectivity.

## MATERIALS AND METHODS

**Construction of Plasmids.** To generate pT7-2PR (Fig. 1), the coding region of HIV-1 PR (strain BH10; ref. 9) was amplified by polymerase chain reaction with *Thermus aquaticus* DNA polymerase (New England Biolabs) as described by the supplier. Two different sets of primers were used and the amplified DNAs were subcloned into pBluescript KS+ (Stratagene). All amplified DNAs and all mutants were sequenced through the entire coding region. One set of primers added an *Nde* I site immediately 5′ of PR and four codons of the linker sequence (Ala, Gly, Ala, Ile) immediately 3′ of the PR coding sequence (plasmid pBS-PRI); the other set added four codons of the linker sequence (Ile, Gly, Gly, Ala) 5′ of the PR sequence and two stop codons immediately 3′ of PR (plasmid pBS-PRII). Both copies of PR were joined by using a common *Pvu* I site in the linker region and were cloned into the *Nde* I/*Eco*RI fragment of pET3 (10). Plasmid pT7-PR was generated from pT7-2PR by deleting the small *Bcl* I fragment (Fig. 1). Mutations were introduced into pBS-PRI and pBS-PRII by oligonucleotide-directed mutagenesis using uridine-substituted single-stranded DNA as described (11). Construction of plasmids pHIVgpII and pHIVFSII (renamed pBR-gpII and -FSII) has been described (12). Plasmids pBR-gp2PR and -FS2PR were generated by inserting the small *Bcl* I fragment from plasmid pT7-2PR into pBR-gpII and -FSII. Construction of the eukaryotic expression vectors will be described elsewhere (K. Mergener and H.-G.K., unpublished data). Briefly, the gpII, FSII, and gp2PR regions were placed under the control of the cyto-

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Abbreviations: HIV, human immunodeficiency virus; CA, capsid protein; NC, nucleocapsid protein; PR, proteinase.

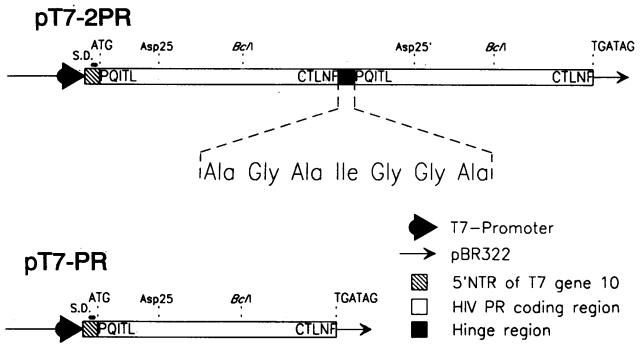


FIG. 1. Structure of the HIV PR gene in the expression plasmids pT7-PR and pT7-2PR. The HIV PR coding region is shown as an open box (N- and C-terminal amino acids are identified by single-letter code), the hinge region is shown as a solid box (amino acid sequence identified beneath), and the untranslated sequence of gene 10 of phage T7 is shown as a hatched box. The T7 promoter (solid triangle), Shine–Dalgarno sequence (S.D.), and start (ATG) and stop (TGATAG) codons are identified. The two active site aspartate codons and the *Bcl* I restriction sites used for generating pT7-PR are also indicated. For clarity the diagram is not to scale.

megalovirus immediate early promoter/enhancer to give pK-R-gpII, -FSII, and -gp2PR. In addition, the plasmids contain the HIV-1 rev responsive element (13) and the simian virus 40 large tumor antigen splice and poly(A) signals. Plasmid pNL43-2PR was constructed by inserting the small *Bcl* I fragment from pT7-2PR into a subclone containing the *Apa* I/*Eco*RI fragment from pNL4-3 (14) and inserting the *Apa* I/*Eco*RI fragment of the resulting plasmid into pNL4-3.

***In Vitro* Transcription and Translation.** Synthetic RNAs were transcribed with T7 RNA polymerase *in vitro* as described (15). RNAs (final concentration, 50 $\mu g/\mu l$) were translated in rabbit reticulocyte lysate (Promega Biotec) in the presence of [$^{35}$S]methionine for 90 min at 30°C as described by the supplier. Cleavage reactions were performed by incubating radioactively labeled translation products with either cleared bacterial extracts or purified HIV PR (16) for 60 min at 37°C as described (16). Relative activities of different expression products were determined by comparing the activity of the extract containing mutant enzyme to serial 1:10 dilutions of the extract containing wild-type enzyme.

**Analysis of Expression Products.** For analysis of HIV antigens, media were cleared for 10 min at 200 × *g* and appropriate dilutions were analyzed with a commercial ELISA kit (Organon Teknika), detecting primarily the capsid (CA) protein. ELISA dilutions were chosen to be in the linear response range of the ELISA. Viral and subviral particles were precipitated from the medium by incubation with 7.5% polyethylene glycol (PEG) 6000/1 M NaCl for 60 min at 0°C. Precipitates were collected by centrifugation at 15,000 × *g* for 15 min and were analyzed by ELISA or Western blot.

## RESULTS

**Expression of a Single-Chain Dimer of HIV PR.** For expression of a single-chain dimer of HIV PR, a plasmid was constructed containing, under control of gene 10 of phage T7, two copies of HIV PR preceded by a methionine codon and linked by a short segment encoding a structurally flexible hinge region (pT7-2PR; Fig. 1). A similar vector containing only a single copy of PR without additional HIV sequences was also constructed (pT7-PR; Fig. 1). Expression of HIV PR as a monomer or as a genetically linked dimer in *Escherichia coli* BL21 (DE3)pLysS (17) yielded protein products of the expected sizes (11 and 23 kDa, respectively) that were absent in bacteria transformed with the vector alone (Fig. 2*A*). The single-chain dimer of HIV PR was stable on prolonged incubation and no immunoreactive band at, or close to, the migration of the monomer could ever be detected. Activity of the expression products was assayed by incubation with a HIV gag polyprotein obtained by translation *in vitro* (Fig. 2*B*; ref. 12). The products from both constructs were shown to be active and the expected cleavage products (as compared to incubation with purified HIV PR; data not shown) were observed. In dilution experiments, we consistently observed a 2- to 5-fold higher activity for the linked dimer.

An aspartate to alanine substitution of the active site (Asp-25) was introduced separately into the first copy (construct 25/1), the second copy (construct 25/2), or both PR

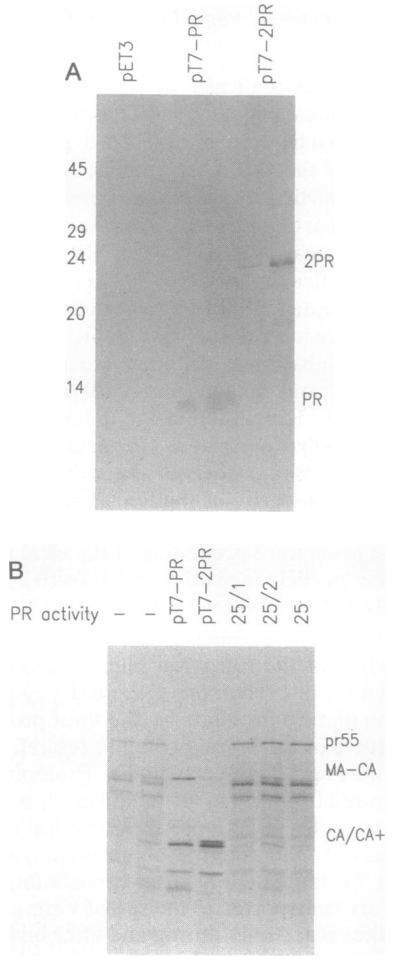


FIG. 2. Analysis of expression products from pT7-PR and pT7-2PR. (*A*) Western blot analysis of induced *E. coli* BL21 (DE3)pLysS (ref. 17; for induction, see ref. 12) transformed with the plasmids indicated. In lanes 2, 4, and 6, 3 times the amount of lysate was loaded as in lanes 1, 3, and 5. Proteins were separated on a 15% polyacrylamide gel and Western blots were stained with a rabbit polyclonal antiserum against PR (gift of P. Barr). Molecular mass standards (in kDa) are indicated on the left; migration of the PR monomer and the linked dimer (2PR) is identified on the right. (*B*) Activity of expression products against *in vitro* synthesized gag substrate. Samples of translation mixtures programmed with gpII RNA were incubated with induced bacterial extracts (see ref. 16) containing the plasmids indicated on top. In the first two lanes, no extracts were added and the reaction was either kept on ice (first lane) or incubated for 60 min at 37°C (second lane). The reactions were separated on a 17.5% polyacrylamide gel and were analyzed by autoradiography. Constructs 25/1, 25/2, and 25 are derived from pT7-2PR but contain an active site aspartate to alanine mutation in the first, second, or both copies of the single-chain dimer. Relevant gag proteins are identified on the right (CA+ indicates heterogeneity in C-terminal processing of CA giving rise to several species of CA with slightly different electrophoretic mobilities; see ref. 16). MA, matrix protein.

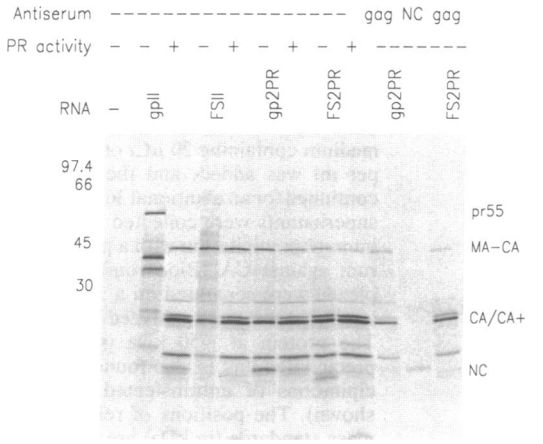


FIG. 3. Analysis of *in vitro* synthesized HIV polyproteins. Samples of translation mixtures, programmed with the RNAs indicated on top, were either analyzed directly (−PR activity) or were incubated with purified HIV PR and then analyzed (ref. 16; +PR activity). In addition, samples programmed with gp2PR and FS2PR RNA were subjected to immunoprecipitation with antiserum against HIV gag proteins (gift of R. Wisniewolski; ref. 12) and a sample programmed with gp2PR RNA was immunoprecipitated with antiserum against HIV NC protein (Biochrom, Berlin) as indicated. All reactions were separated on a 17.5% polyacrylamide gel and were analyzed by autoradiography. MA, matrix protein.

copies (construct 25) of the single-chain dimer. Expression of pT7-2PR(25/1) and pT7-2PR(25) yielded completely inactive products, whereas the activity of the heterodimer containing an active site mutation in the second PR copy (25/2) was reduced by a factor of $>2 \times 10^3$ (Fig. 2*B*). Mixing of the two heterodimers (25/1 and 25/2) did not restore activity (data not shown), indicating that only the linked dimer, and not multimers thereof, contained the proteolytic activity.

**Translation of HIV Polyproteins.** HIV PR is encoded within the viral *pol* gene and synthesis of the enzyme requires a translational frameshift event to yield a gag-pol polyprotein (18). Translation *in vitro* of synthetic RNAs containing the HIV gag and PR regions in different reading frames has been shown to yield stable polyproteins (Fig. 3, lanes gpII; ref. 12), whereas translation of gag and PR in the same reading frame (facilitated by a short insertion close to the normal frameshift site) gave rapid autocatalytic processing (Fig. 3, lanes FSII; ref. 12). When the single-chain dimer of HIV PR was present on *in vitro* synthesized HIV polyproteins, efficient and specific autocatalytic processing was observed even when gag and PR were in different reading frames (Fig. 3, lanes gp2PR). The processing pattern was indistinguishable from that observed after incubation of the gag polyprotein ($pr55^{gag}$) with purified HIV PR (Fig. 3, compare lanes gp2PR −PR activity to gpII +PR activity). No difference in the processing pattern was observed when gag and the genetically linked PR copies were placed in the same reading frame (Fig. 3, compare lanes gp2PR and FS2PR) and the same products could be immunoprecipitated by using an antiserum directed against the gag proteins (Fig. 3). The only difference was the presence of the nucleocapsid (NC) protein after translation of gp2PR RNA (Fig. 3), which is absent from FSII and FS2PR translation reactions because of the insertion and subsequent change to the pol reading frame in the NC region. Incubation of the translation products from gp2PR RNA, FS2PR RNA, and FSII RNA with 20 nM purified HIV PR did not give additional processing of the intermediate products (Fig. 3, lanes +PR activity), whereas the addition of higher concentrations of PR gave complete processing (data not shown).

**Effect of PR Single-Chain Dimer on Particle Assembly.** The region encoding the single-chain PR dimer was inserted into the full-length proviral cDNA (pNL4-3, ref. 14; pNL43-2PR). In addition, the viral gag and PR regions (either wild type or with the linked dimer of PR) were cloned into a eukaryotic expression vector (plasmids pK-R-gpII and pK-R-gp2PR, respectively). HIV-specific protein synthesis was analyzed by ELISA 48 hr after electrotransfection (19) of the different constructs into COS-7 cells. Table 1 shows that both pNL4-3 and pNL43-2PR transfected cells released comparable amounts of HIV antigen into the medium. However, when particles were separated from nonparticulate antigen [either by PEG precipitation or by centrifugation through a sucrose cushion (data not shown)], no HIV antigen was detected in the precipitate from pNL43-2PR transfected cells, whereas 50% of the total released antigen could be recovered in the precipitate in the case of PNL4-3 (Table 1). Supernatants from transfected COS-7 cells were incubated with the $CD4^+$ lymphocytic cell line MT-4 (20) to assay for the production of infectious virus. Replicated HIV could be readily detected after 3 days for the wild-type construct pNL4-3 (Table 1), and after 6 days, the MT-4 culture was >80% infected. No infectious virus could be recovered from pNL43-2PR trans-

Table 1. Analysis of HIV antigens released after transfection and infection

| Plasmid(s) | COS-7 48 hr posttransfection, RAU/ml | PEG precipitated/total antigen, % | MT-4 3 days postinfection, RAU/ml | MT-4 6 days postinfection, RAU/ml |
|---|---|---|---|---|
| Mock | 0 | — | 0 | 0 |
| pNL4-3 | 5500 | 50 | 11,000 | 18,000 |
| pNL43-2PR | 4200 | 0 | 50 (input, 40) | 180 (input, 200) |
| pK-R-gpII + pMTcrev | 1700 | 20 | ND | ND |
| pK-R-gp2PR + pMTcrev | 760 | 0 | ND | ND |

Supernatants from COS-7 cells transfected with the plasmids indicated were collected 48 hr after transfection and analyzed for the release of HIV antigens by ELISA. After PEG precipitation of medium, the relative amount of precipitated HIV antigen (measured by ELISA) is given as a percentage of the total HIV antigen in the medium. Cleared supernatants from transfected COS-7 cells were mixed with fresh MT-4 (20) cells at a ratio of 1:9 and incubated for 3 days or were mixed at a ratio of 1:1 and incubated for 6 days postinfection. Subsequently, supernatants were analyzed for HIV antigens by ELISA. Each value represents the average of at least three experiments using at least two batches of DNA. All individual results were in the range of ±25% of the average value. Antigen concentrations are given as relative absorbance units (RAU)/ml, an arbitrary unit derived from the absorbance readings, with 1000 RAU/ml approximately equivalent to a CA protein concentration of 100 ng/ml. The same positive control was used in all experiments to allow standardization of the results. ND, not determined.

fected cells even after prolonged periods of incubation with the reporter cell line (Table 1). Moreover, even 6 days after infection, the same amount of ELISA antigen was detected in the supernatant of MT-4 cells as was initially used for infection (Table 1), indicating that no uptake of HIV-specific antigens had taken place.

Release of particles was also analyzed after transfection of the expression vectors pK-R-gpII and pK-R-gp2PR into COS-7 cells. As in the case of the respective proviral constructs, no HIV-specific antigen could be PEG-precipitated from the medium of pK-R-gp2PR transfected cells, whereas in the case of the wild-type construct, 20% of the total antigen in the medium was precipitable (Table 1). Western blot analysis of the precipitate yielded the pr55$^{gag}$ precursor, an intermediate containing matrix protein and CA and the mature CA protein, whereas no HIV-specific proteins were observed in the case of pK-R-gp2PR [Fig. 4; similar results were obtained for pNL4-3 and pNL43-2PR (data not shown)]. Extracts from pK-R-gp2PR transfected cells contained considerably less cell-associated gag proteins than in the case of pK-R-gpII transfected cells (Fig. 4). These proteins were, however, completely processed because of the presence of the genetically linked PR dimer on the polyprotein [Fig. 4; similar results were obtained for pNL43-2PR (data not shown)].

The supernatants of [$^{35}$S]methionine-labeled, transiently transfected COS-7 cells were analyzed by immunoprecipitation with an antiserum against CA. The CA antigen could be detected in the medium independent of the formation of viral or subviral particles (Fig. 5) but, in contrast to the ELISA data, considerably less CA protein was immunoprecipitated from the medium of pNL43-2PR transfected cells when compared to the wild-type proviral construct (Fig. 5 and Table 1). This is due to the fact that labeling of transfected cells was performed from 24–60 hr after transfection,

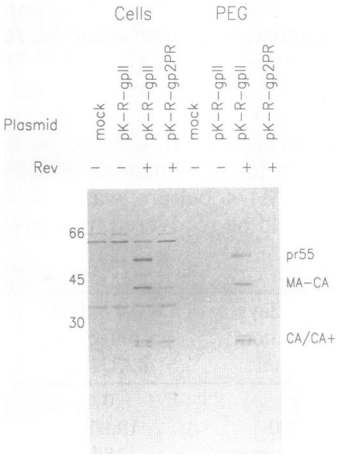


FIG. 4. Transient expression of HIV sequences in COS-7 cells. Approximately $5 \times 10^6$ subconfluent cells were electrotransfected at 150 V with the plasmids indicated (10 or 20 $\mu$g of DNA). The other parameters were as described (19). Rev + indicates that cotransfection with the rev expression vector pMTcrev (gift of B. Felber and G. Pavlakis; ref. 21) was performed. The presence of rev responsive element in cis on the RNA and of the rev protein in trans is required for expression of HIV structural genes in eukaryotic cells (ref. 13; compare lanes pK-R-gpII −rev and +rev). Cells were harvested 48 hr after transfection and lysates were analyzed by electrophoresis on a 17.5% polyacrylamide gel and Western blotting [lanes 1–4 (Cells)]. Supernatants from transfected cells were harvested at 48 hr, precipitated with PEG, and analyzed by Western blotting [lanes 5–8 (PEG)]. The blot was stained with a polyclonal antiserum against gag proteins (gift of R. Wisniewolski; ref. 12). Molecular mass standards (in kDa) are shown on the left; relevant gag proteins are identified on the right. MA, matrix protein.

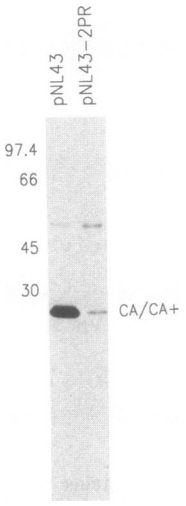


FIG. 5. Immunoprecipitation of labeled supernatants from transfected cells. COS-7 cells were transfected with the plasmids indicated. Cells were washed 24 hr after transfection, medium containing 20 $\mu$Ci of [$^{35}$S]methionine per ml was added, and the incubation was continued for an additional 36 hr. Radiolabeled supernatants were collected and subjected to immunoprecipitation with a polyclonal antiserum against CA (Biochrom). Immune complexes were separated on a 17.5% polyacrylamide gel and were analyzed by fluorography. The protein at ≈50 kDa is nonspecifically precipitated and is also found in immunoprecipitations of untransfected cells (data not shown). The positions of relevant molecular mass standards (in kDa) are indicated on the left; the position of the HIV CA(s) is identified on the right.

whereas for the ELISA, medium was collected 48 hr after transfection without change of medium. It was determined that early (6 hr) after transfection with pNL4-3 and pNL43-2PR, ≈1% of the total cells were fluorescence positive in both cases. While the number of positive cells increased in both cases by 24 hr after transfection, a considerable proportion of cells transfected with pNL43-2PR had already lost their normal appearance. Late (48 hr) after transfection with pNL43-2PR there were virtually no normally shaped fluorescence-positive cells but abundant strongly fluorescent round cell "ghosts." Double staining of these coverslips with fluorescent antibody and the DNA strain 4′,6-diamidino-2-phenylindole dihydrochloride (DAPI) revealed that all fluorescence-positive cells late after transfection had lost their nuclear integrity and exhibited weak and irregular DAPI fluorescence throughout the cell (data not shown). Conversely, 48 hr after transfection of pNL4-3, there were abundant strongly immunofluorescence-positive cells (5–15% of total cells) with normal shape (data not shown).

The observed phenotype of the 2PR constructs was dependent on the translation of the pol open reading frame and was completely abolished by a single nucleotide deletion in pNL43-2PR, which leads to premature termination upstream of the start of the pol open reading frame (data not shown).

## DISCUSSION

In this communication, it has been shown that a stable active single-chain dimer of HIV PR can be generated by covalently linking two copies of PR by a short flexible hinge region. Since the two PR copies are cloned in separate mutagenesis vectors, mutations can be introduced separately into both copies and the two possible heterodimers as well as the homodimer can easily be generated. Using this mutagenesis approach, it was shown that only the genetically linked dimer, and not multimers thereof, contains the proteolytic activity and that both aspartate residues of the single chain dimer are required to generate a competent active site. These experiments also show the potential of this system to produce stable heterodimers of HIV PR in order to evaluate the contribution of specific amino acids toward the stability and activity of PR. These results agree with recent experiments by DiIanni *et al.* (22), who showed that a single-chain dimer of HIV PR with a glycine-glycine hinge region was active against HIV substrates and had similar kinetic parameters when compared to PR expressed as a monomer. These authors also confirmed that the aspartate residues from both PR copies are required for PR activity (22).

Expression of the single-chain dimer as a component of the viral polyprotein led to rapid autocatalytic processing after

translation *in vitro*, indicating that the lack of cleavage observed in previous translation experiments with wild-type sequences (12) was in fact due to the low concentration of PR dimer. The presence of a fully competent aspartic PR on the polyprotein allows for the synthesis of active PR independent of the polyprotein concentration, which in *in vitro* translation experiments may not be sufficient for intermolecular dimerization to occur.

Consistent with the *in vitro* translation experiments, expression of the dimeric PR as a component of the viral gag-pol polyprotein after transfection of proviral and subviral constructs also led to rapid premature processing, thus preventing particle assembly. Premature processing may normally be prevented by inefficient dimerization due to the low concentration of gag-pol polyproteins in the cytoplasm, and concentration of these polyproteins at the site of assembly may therefore be an important factor for the initiation of processing. Our results do not rule out the possibility that a cellular activity (proteinase or cofactor) is required for initial release of the enzyme from the polyprotein or that cellular or viral factors prevent the premature activation of dimerized polyproteins. However, these processes are either not rate limiting or are not functioning once a dimeric enzyme is present on the polyprotein.

An increased cytotoxicity was observed in all instances when premature processing occurred, and 48 hr after transfection no viable cells expressing the HIV antigens were found. Taken together with the results of Western blots of transfected cells, analysis of cell-free antigen by ELISA, and radioactive labeling of transfected cells, these results indicate that early after transfection with the 2PR constructs, normal synthesis of HIV-specific proteins takes place. However, the resulting polyproteins immediately undergo autocatalytic processing and either the PR dimer or any of the polyprotein cleavage products may be toxic to the cells, leading to rapid cell death and subsequent release of viral antigens into the supernatant. After 24 hr, most of the transfected cells are no longer capable of normal protein synthesis, although release of viral antigens still continues due to lysis of dead cells. The recent observation that purified HIV PR can induce cleavage of components of the cytoskeleton *in vitro* (23) suggests that the observed cytotoxicity may be directly caused by the proteolytic activity of the single-chain PR dimer.

Polyprotein processing is an early event in the replication of plus-strand RNA viruses, whereas the cleavage of retroviral polyproteins occurs late, concomitant with or subsequent to particle assembly (2). These differences in the timing of proteolysis may reflect the different modes of replication. In both viral systems processing is required, on the one hand, to separate and activate functionally distinct domains (e.g., polymerases) and, on the other hand, it is needed for the conversion of preassembled nucleocapsids, thereby leading to the maturation of infectious virions. However, plus-strand RNA viruses require immediate separation of different domains to allow replication to proceed and the replicative proteins are not packaged into the virion. Conversely, retroviral polyproteins are translated only after transcription of integrated proviral DNA and they are not immediately used for replication but are packaged into the virion, and replication occurs in the newly infected cell. Thus, the presence of an active or an initially inactive PR on polyproteins from different viruses may be dictated by their particular mode of replication, which, in the case of retroviruses, requires the PR to be inactive until all virion components are confined in the budding particle.

Tight regulation of proteinase activity appears to be critical for successful HIV replication since premature processing would dissociate the components of the virion from the site of assembly and incomplete or aberrant cleavages should interfere with uncoating and replication. Thus, not only inhibition of HIV PR, leading to noninfectious particles (24), but also premature activation of PR, preventing particle assembly and causing cell death, is detrimental to the virus.

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