Human immunodeficiency virus type 1 Vif-derived peptides inhibit the viral protease and arrest virus production

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Received 20 October 1998; received in revised form 24 November 1998

Abstract Human immunodeficiency virus type 1 (HIV-1) Vif protein is required for productive HIV-1 infection of peripheral blood lymphocytes and macrophages in cell culture and for pathogenesis in the SCID-hu mouse model of HIV-1 infection. Vif inhibits the viral protease (PR)-dependent autoprocessing of truncated HIV-1 Gag-Pol precursors expressed in bacterial cells and efficiently inhibits the PR-mediated hydrolysis of peptides in cell-free systems. The obstructive activity of Vif has been assigned to the 92 amino acids residing at its N′-terminus (N-Vif). To determine the minimal Vif sequence required to inhibit PR, we synthesized overlapping peptides derived from N-Vif. These peptides were then assessed, using two in vitro and two in vivo systems: (i) inhibition of purified PR, (ii) binding of PR, (iii) inhibition of the autoprocessing of the Gag-Pol polyprotein expressed by a vaccinia virus vector, and (iv) inhibition of mature virus production in human cells. The peptides derived from two regions of N-Vif encompassing residues Tyr-30–Val-65 and Asp-78–Val-98, inhibited PR activity in both the in vitro and the in vivo assays. Thus, these peptides can be used as lead compounds to design new PR inhibitors.

Key words: HIV-1 protease; Protease regulation; Peptide; Vif; Human immunodeficiency virus type 1; Acquired immunodeficiency syndrome

1. Introduction

Vif (virion infectivity factor), one of the auxiliary proteins expressed by human immunodeficiency virus type 1 (HIV-1), is conserved among HIV-1 isolates and in lentiviruses [1–4]. In the absence of Vif, HIV-1-infected non-permissive cells produce non-infectious virions [5–10] that penetrate cells but are unable to efficiently synthesize viral DNA [10–12]. Vif-deficient particles can effectively initiate virion production only in certain lymphoid cell lines, such as SupT 1, which are able to complement Vif function(s) [13,14]. Vif-deficient viruses which are produced by peripheral blood lymphocytes (PBL) or macrophages, the two major HIV-1 target cells in the human body, have aberrations in core protein composition [6,14] and in core morphology [6,15]. Virions carrying partially processed Gag and Gag-Pol precursors display attenuated infectivity [14], suggesting that Vif ensures the proper processing and assembly of viral precursors required for the production of infectious virions. Support for this notion was recently provided by Bouyac et al. [16], who demonstrated that sequences at the half C′-terminus of Vif (C-Vif) interact with two regions of the HIV-1 Gag protein.

The retroviral Gag and Gag-Pol polyproteins are translated from mRNA that is indistinguishable from the full-length genomic RNA found in virions. The autoprocessing of Gag and Gag-Pol precursors into structural and catalytic proteins is carried out by viral protease (PR) [17,18]. The proteolytic activity of PR is essential to the production of infectious viruses [17–19]. Thus, PR has been the target for several antiviral compounds, such as saquinavir, indinavir, ritonavir and nelfinavir, which show therapeutic promise [20].

The HIV-1 PR, enzymatically active as part of the viral precursor [21–23], is tightly regulated: overexpression [24], expression of PR monomers in tandem [25–27], expression of mutated overactivated enzyme [28], or activation of PR prior to virus assembly [27] prevents the generation of infectious particles. Thus, Vif appears to act in non-permissive cells as a proteolytic regulator directing the initiation of precursor processing to the right location, at the correct time, to assure the production of mature virions. Our previous results [29] clearly show that Vif and its N′-terminus (N-Vif; i.e. Vif 1–92), but not C-Vif (i.e. Vif 93–192), inhibit the autoprocessing of truncated Gag-Pol polyproteins expressed in bacteria and that Vif and N-Vif inhibit the hydrolysis of synthetic peptides in vitro [29]. In the present study we performed a peptide mapping of the N-Vif and revealed that the active domains in Vif encompassing residues Tyr-30–Tyr-44 and Asp-78–Val-98. Our results show that peptides derived from these regions inhibited PR activity in both the in vitro and in vivo assays and reduced infectious virus production by HIV-1-infected cells. Thus, we suggest that peptides derived from these regions can be used as lead compound to design new PR inhibitors.

2. Materials and methods

2.1. Cells and viruses

Hut 78 cells and PBL were maintained in RPMI 1640 and HeLa CD4+ cells were maintained in Dulbecco's modified Eagle's medium (DMEM). Media were supplemented with 10% fetal calf serum, antibiotics (penicillin and streptomycin), and 2 mM glutamine. PBL were stimulated with 1 μg/ml of phytohemagglutinin (PHA) 24 h before infection. HIV-1_{IIIB} was kindly supplied by Dr. Wainberg (Lady Davis Institute, Montreal, Canada) and the vaccinia vector (vVK-1), which expresses the entire HIV-1 *gag* and *pol* genes [30], was obtained from Dr. B. Moss (Laboratory of Viral Diseases, NIAID, NIH, Bethesda, MD, USA).

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PII: S0014-5793(98)01602-0

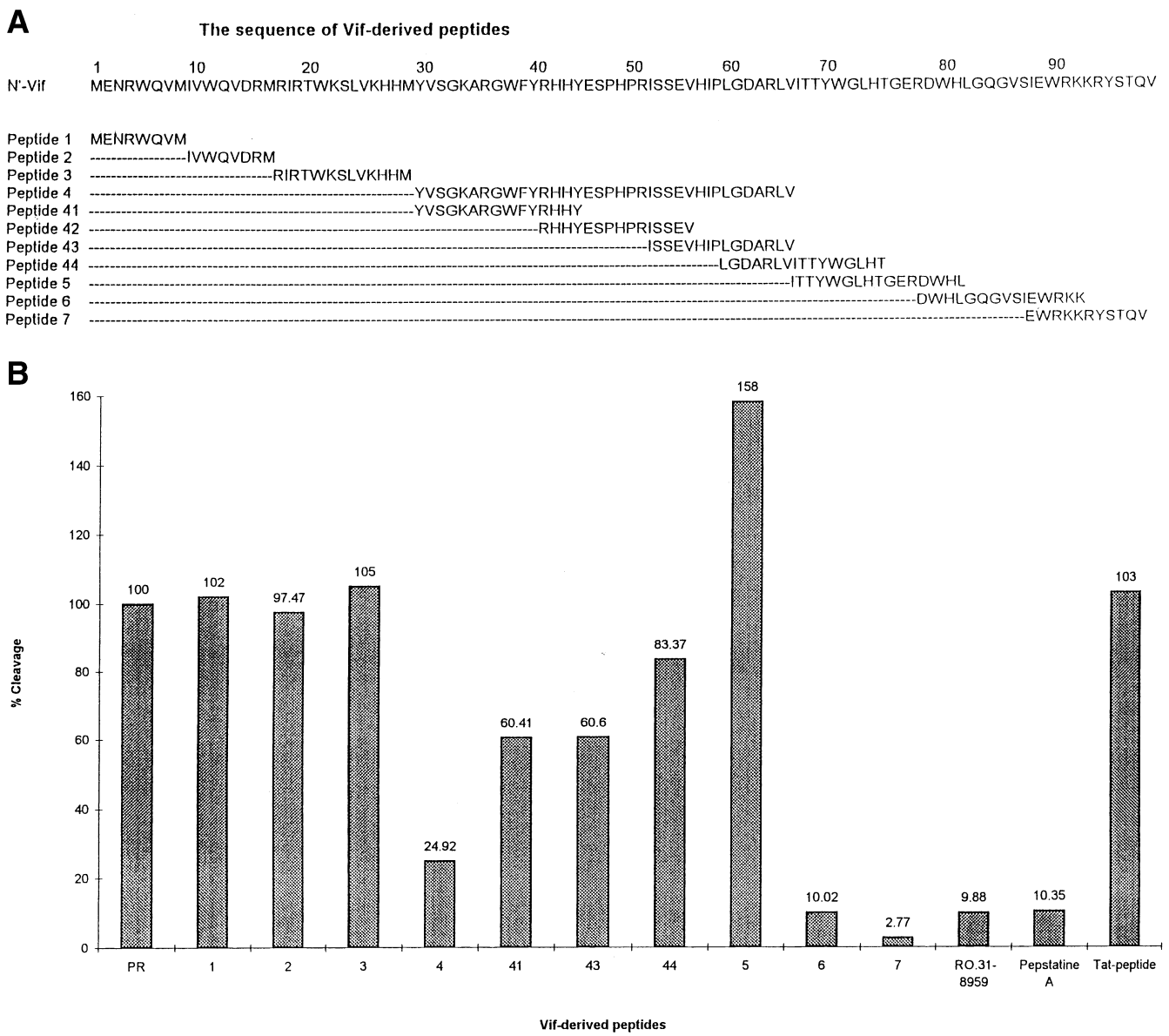

Fig. 1. A: Sequence of peptides derived from the N′-terminus of the HIV-1 Vif protein (upper row) that were synthesized and tested in this study. B: Screening of Vif-derived peptides for inhibition of PR-mediated hydrolysis of synthetic peptide substrate in vitro. Hydrolysis of decapeptide substrate by HIV-1 PR (0.5 μM) in the presence of Vif-derived peptides (1 mM), pepstatin A or Ro31-8959 (0.1 mM), HIV-1 Tat-derived peptide Cys-Gly-Arg-Lys-Lys-Lys-Arg-Arg-Gln-Arg-Arg-Arg-Ala-His-Gln-Asn (1 mM) was analyzed by HPLC (peptide 42 overlapped with the substrate peak and could therefore not be assayed). The cleavage products of substrate in the standard reaction without inhibitors (36%) is taken as 100%. C: Dose-dependent inhibition of PR activity by Vif-derived peptides. Reactions were carried out with increasing concentrations of Vif-derived peptides in standard reaction mixtures where the uninhibited reactions (25% cleavage) were taken as 100%. The data in B and C are from one of three experiments with similar results.

2.2. Titration of HIV-1 on HeLa CD4+ cells

HeLa CD4+ cells plated on 96 wells were infected with 20 μl of clarified medium harvested from HIV-1-infected Hut 78 cells. Following absorption for 60 min at 37°C, the virus was replaced with fresh medium. To determine the number of LacZ-positive cells, cultures were fixed with 2% formaldehyde and stained with X-Gal as previously described [31].

2.3. Analysis of viral proteins

Twenty four hours post infection with vVK-1 at a multiplicity of infection (MOI) of 1, cells were pelleted at 2000 rpm for 5 min. The supernatants were cleared of cell debris by spinning at $10\,000 \times g$ for 10 min, before centrifugation for 45 min at $100\,000 \times g$ in a Beckman centrifuge (Ti-50.2 rotor) through a 20% sucrose cushion. Cellular and particle pellets were dissolved in Laemmli buffer, separated by electrophoresis before Western blotting. Blots were incubated with monoclonal antibody against HIV-1 CA (contributed by Dr. K. Steimer and obtained through the AIDS Research and Reference Reagent Program, Division of AIDS, NIAID, NIH) and visualized by enhanced chemiluminescence (Sigma). In each experiment equal numbers of vVK-1-infected cells were used to prepare lysates.

Five days post infection of Hut 78 cells with HIV-1_{IIIB} at an MOI of 0.5, cells were washed and incubated for 1 h with a mixture of 1 ml of RPMI 1640 medium and 0.5 ml water containing 1 mg peptide. Following incubation, cell suspensions were diluted with RPMI 1640 medium to a final concentration of 60 μM peptide and incubated for an additional 48 h. Cells and viruses were harvested and analyzed as described above.

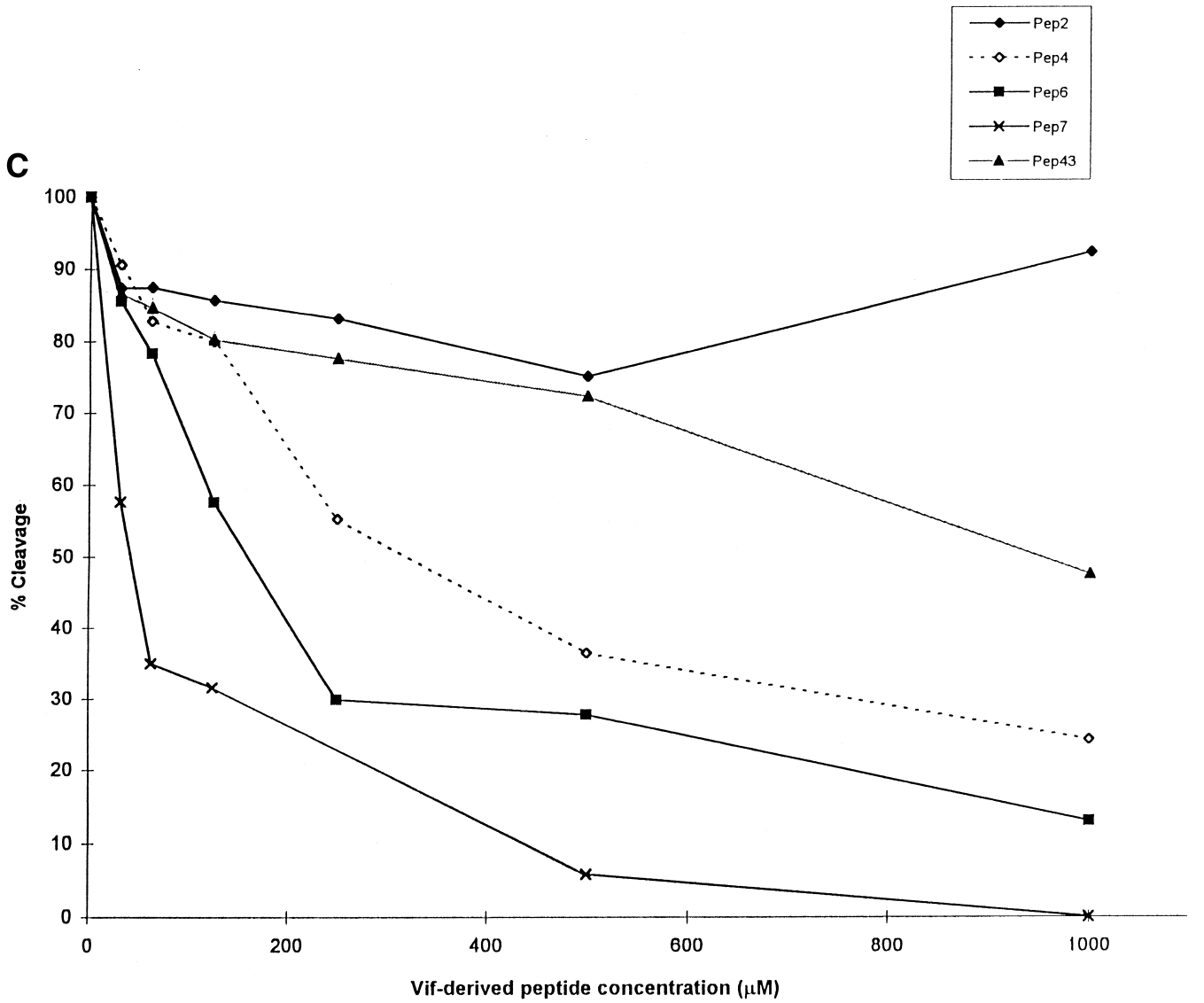

Fig. 1. (*continued*)

2.4. Peptides

Peptides were synthesized according to the SPPS method, using an Applied Biosystems peptide synthesizer, model 433A on Rink amide resin (loading 0.5 mmol/g) by standard Fmoc chemistry. They were cleaved from the resin with trifluoroacetic acid (TFA) containing 5% anisole as a scavenger, precipitated from cold ether, dissolved in water and lyophilized. Crude peptides were analyzed by reverse-phase HPLC (C3 column 5–60% acetonitrile: water gradient containing 0.1% TFA, 45 min) and characterized by TOF-MS and amino acid analysis.

2.5. Synthetic peptide cleavage assay

The standard conditions of the assay were essentially as described previously [32,33]. A standard reaction mixture (10 μl) contained 1 mM of the natural MA/CA junction decapeptide (Val-Ser-Gln-Asn-Tyr-Pro-Ile-Val-Gln-Asn), 0.5 μM purified PR [22] and 0.1 M NaCl in 50 mM sodium phosphate buffer (pH 5.5). Mixtures were incubated at 37°C and the reaction was terminated by the addition of 90 μl guanidine-HCl (pH 8.0) to a final concentration of 6 M. Aliquots of 80 μl were analyzed by reverse phase HPLC (Vydac C_{18} column; 0–40% acetonitrile:water gradient containing 0.1% TFA) and percent cleavage was calculated as the ratio between the peak areas corresponding to the cleavage product (P) and uncleaved substrate (S).

$$\% \text{ Cleavage} = [P/(P+S)] \times 100$$

The cleavage of substrate obtained in standard reaction mixtures without inhibitors was taken as total uninhibited protease activity, represented by 100% cleavage.

2.6. Vif-derived peptide/PR binding assay

Each well of a 96 microwell ELISA plate (M-129B, Dynatech) was coated for 18 h at 4°C with 200 μl of a solution containing 12 μM peptides in 100 mM Tris-HCl (pH 8.8). This amount of peptide was sufficient to saturate the wells. In order to stabilize peptide 7, dithiothreitol (1 mM) was added to all solutions containing this peptide. The wells were aspirated, incubated with low fat milk for 1 h and washed with PBS containing 0.05% Tween 20. A volume of 200 μl containing 20 nM PR in 0.1 M NaCl and 50 mM sodium phosphate buffer (pH 7.4) was then added to each well and the microplates were incubated for 2 h at room temperature.

In the competitive binding experiments, 20 nM of PR (in 200 μl of 100 mM Tris-HCl pH 8.8) was incubated for 18 h at 4°C with 14 nM Ro31-8959, with 60 μM of each peptide, or with 25 μM of peptide 4

before being added to the coated wells. The plates were extensively washed and PR binding was determined using rabbit anti-PR serum and alkaline phosphatase-conjugated goat anti-rabbit IgG diluted 1:1000 (Sigma) as secondary antibody. The wells were developed by adding substrate *p*-nitrophenyl phosphate (Sigma) and bound PR was quantified with an ELISA reader (Dynatech MR5000) at 405 nm.

3. Results

3.1. Vif-derived peptides inhibit PR activity

To determine which active Vif domain(s) is capable of inhibiting PR, a set of partially overlapping peptides was synthesized, based on the sequence of HIV-1 BH10 Vif [34] (Fig. 1A). Inhibition of HIV-1 PR activity by Vif-derived peptides was determined by the reduction of cleavage product (P) formation in a standard reaction mixture in comparison with uninhibited PR. Screening of crude peptides (70–90% purity) for PR inhibition revealed that peptides derived from two regions of Vif (30–65 and 78–98), namely peptides 4, 6 and 7, inhibited proteolysis (Fig. 1B). The IC_{50} of peptide 4 was in the range of 230–250 μM, whereas the IC_{50} values of peptides 6 and 7 were in the order of 110 and 25 μM, respectively (Fig. 1C). The 11 amino acids long peptide 7 also inhibited the cleavage of the fluorogenic substrate Arg-Glu(EDANS)-Ser-Gln-Asn-Tyr-Pro-Ile-Val-Gln-Lys(DABCYL)-Arg (50 μM) by HIV-1 PR and the IC_{50} value was 3.3 μM (results not shown). Peptides 41 and 43, each composed of 15 amino acid residues, were less active than peptide 4 (Fig. 1B). Overlapping peptides 6 and 7, containing the Arg-Lys-Lys-Arg motif, proved to be efficient inhibitors.

3.2. Binding of HIV-1 PR to Vif-derived peptides

The interaction of Vif-derived peptides with PR was tested by ELISA, using rabbit anti-PR (prepared by immunizing rabbits with purified PR expressed in bacteria). Microtiter plates were coated with peptides and PR was then added with or without preincubation with the cognate peptide. Peptides 4, 41, 42, 43, 6, and 7, but not peptide 3, significantly bound PR. Binding of PR to the absorbed peptides, except peptide 42, was specifically inhibited by preincubation with the cognate peptides (Fig. 2A). There was no specific interaction between PR and peptides 2 and 44 (results not shown).

The concentration of PR required to saturate the absorbed peptides was 6–10 nM (Fig. 2B). To determine the concentration of peptide required to block binding of PR to the absorbed peptide, the enzyme (10 nM) was preincubated with increasing concentrations of cognate peptide. Binding of PR to peptides 4, 41, 43, 6 and 7 was specific, as preincubation of the enzyme with each of the cognate peptides resulted in dose-dependent inhibition (Fig. 2C). In contrast, the binding of PR to peptide 42 was not affected, indicating again that the interaction was non-specific.

The specific PR inhibitor Ro31-8959 [35], which blocks the active site of PR, was used as a control. Preincubation of PR with the inhibitor reduced the binding of the enzyme to Vif-derived peptides (Fig. 2A), showing the specificity of the reaction.

3.3. Inhibition of processing of Gag-Pol precursors expressed by vVK-1 in human cells

We analyzed the effect of Vif-derived peptides on the processing of HIV-1 precursors expressed in human cells infected with vVK-1 [30]. Infection of human T-cell line Hut 78 and

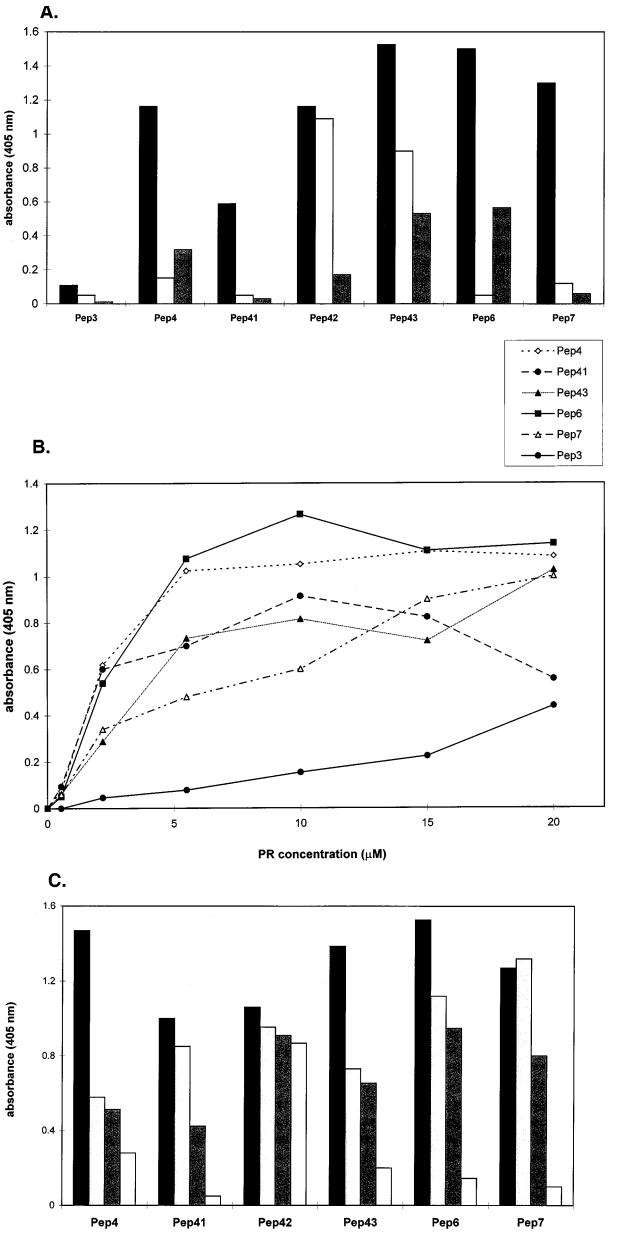

Fig. 2. A: Competitive binding of purified PR to Vif-derived peptides, determined by ELISA. Binding of PR (20 nM) to the Vif-derived peptides (black bars), binding with competing peptide 4 (25 μM) and peptides 3, 41, 42, 43, 6 and 7 (60 μM) (white bars), or Ro31-8959 (14 nM) (gray bars). B: Dose-dependent binding of PR to Vif-derived peptides. The assay was carried out as described in A with increasing concentrations of PR. C: Competitive inhibition of PR binding by Vif-derived peptides. 10 nM PR was preincubated for 18 h in 200 μl with increasing concentrations of the cognate peptides before adding the mixture to the peptide-coated microwells. Black bars: without peptide; vertically hatched bars: 0.6 μM; gray bars: 6 μM; white bars: 30 μM for all peptides, while the concentrations of peptide 4 were 0, 0.25, 2.5 and 12.5 μM, respectively.

PHA-stimulated PBL with vVK-1 resulted in high levels of Gag protein production within 24 h, allowing the natural PR-mediated processing of viral precursors and the release of virus-like particles. As shown in Fig. 3, vVK-1-infected cells and the particles exported to the culture medium by these cells contained completely or partially processed Gag proteins, namely $p55^{Gag}$, MA-CA p41 and CA p24, as determined

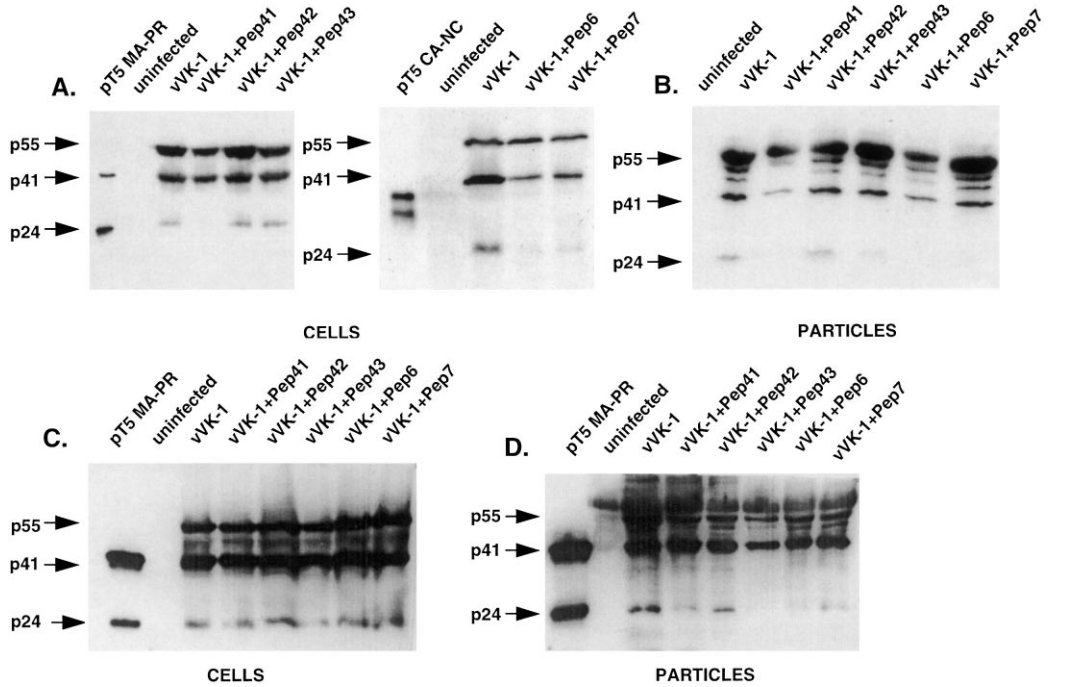

Fig. 3. Inhibition of autoprocessing and of virus-like particle maturation by Vif-derived peptides. Hut 78 cells (A and B), and PBL (C and D) were infected with vVK-1 expressing HIV-1 Gag-Pol and cultured in the presence of the respective Vif-derived peptides. Cells (A and C) and extracellular virus-like particles (B and D) were harvested 24 h post infection and analyzed by Western blotting for the presence of Gag-containing proteins, using monoclonal anti-CA antibody. Incubation of the cells with 60 μM of the peptides did not cause any cytotoxic effect, as determined by trypan blue staining. The pT5 lanes contain a bacterial lysate from cells expressing HIV-1 MA to PR or CA to NC [21,22,29]. The mobility of $p55^{Gag}$, p41 including MA and CA, and p24 CA is indicated to the left of each autoradiogram.

by Western blot. vVK-1-infected Hut 78 cells incubated for 24 h with peptides 42 and 43 contained the same HIV-1 polyproteins present in the control vVK-1-infected cells. However, cells incubated with peptides 41, 6 and 7 contained the unprocessed MA-CA and $p55^{Gag}$ polyproteins and only small amounts of mature CA protein (Fig. 3A). The ratio of $p55^{Gag}$ to MA-CA p41 proteins in cells treated with peptides 6 and 7 is higher than the $p55^{Gag}$/p41 ratio in vVK-1 cells, suggesting that these peptides, as well as peptide 41, inhibit the autoprocessing of viral precursors.

Production and autoprocessing of HIV-1 precursors in vVK-1-infected PBL were not dramatically affected by the Vif-derived peptides (Fig. 3C). However, particles released from PBL (Fig. 3D) and from Hut 78 cells (Fig. 3B) following treatment with peptides 41, 6 and 7, contained mostly unprocessed Gag polyproteins and only minor fractions of mature CA protein. Peptide 43 exerted an effect similar to that of peptides 41, 6, and 7 on particles released from PBL (Fig. 3D), but did not affect, or only slightly affected, the particles released from Hut 78 cells. These results clearly demonstrate that peptides derived from residues Tyr-30–Tyr-44 and Asp-78–Val-98 inhibit the processing of the viral precursors ex-

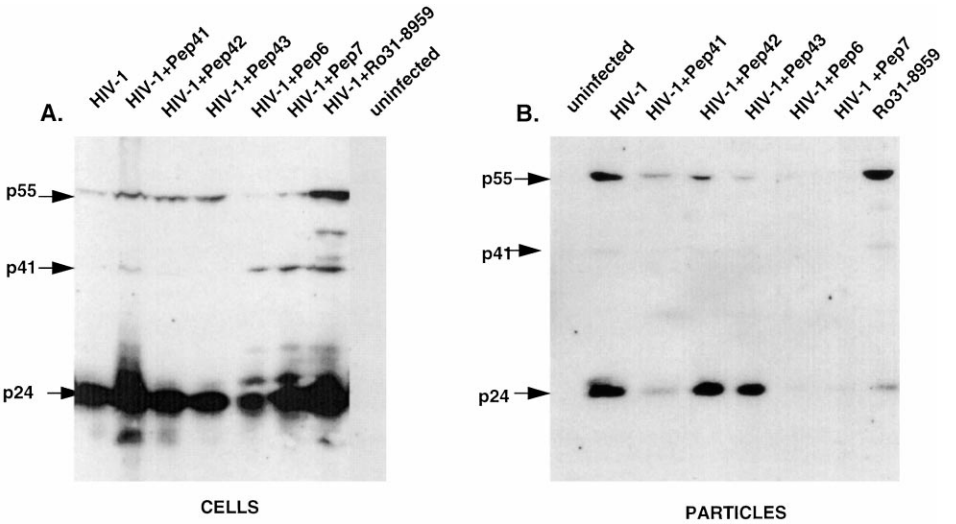

Fig. 4. Inhibition of HIV-1 maturation by Vif-derived peptides. Hut 78 cells (A) and extracellular virions (B) were harvested as described in Fig. 3 and analyzed by Western blotting using monoclonal anti-CA antibody.

pressed in human cells. Differences in penetration and stability of the linear Vif-derived peptides may have caused the various effects on viral precursor processing observed in the cells types used. The inhibition of viral precursors autoprocessing exerted by peptides 41, 6 and 7 cannot account for their toxicity on the host cells because similar amounts of viral proteins are accumulated in treated and untreated cells (Fig. 3A,C).

3.4. *Vif-derived peptides reduce the production of infectious virus*

The effect of Vif-derived peptides on HIV-1 maturation was assessed by incubating Hut 78 cells with the peptides for 48 h, 5 days post infection with HIV-1_{IIIB}. Treatment of cells with peptides 42 and 43 did not influence the autoprocessing of Gag polyproteins, as determined by Western blot of the cell lysates (Fig. 4A). On the other hand, peptides 41, 6 and 7 halt the processing of the Gag precursor in the treated cells as p41 MA-CA and CA-p2 (p26) fusion proteins are clearly detected in the Western blot. However, the cleavage inhibition mediated by Vif-derived peptides was not as efficient as the inhibition by Ro31-8959 since unprocessed $p55^{Gag}$ protein was not observed in these cells (Fig. 4A). Unlike Ro31-8959 treatment which allows the release of particles with uncleaved Gag proteins, peptides 41, 6 and 7, but not peptides 42 and 43, reduced the release of particles containing CA into the culture medium (Fig. 4B). It is possible that the partial inhibition of polyprotein processing reduced or prevented the release of sedimented particles into the medium.

The reduced amounts of p24 CA antigen in the culture media following a 48 h treatment with peptides 41, 6 and 7 (Fig. 4) correlates with the decreased production of infectious virions as titered on HeLa CD4+ cells [31]. Peptides 41, 6 and 7 reduced the titer of infectious virus by 90, 96 and 97%, respectively (Table 1). Thus, we suggest that these peptides interfere with the autoprocessing of viral precursors in human cells infected with HIV-1 and cause a significant reduction in the number of infectious virions released from the HIV-1-infected cells.

Two approaches were taken to determine whether prolonged treatment by Vif-derived peptides reduces virus production. Five days post infection Hut 78 cells were cultured for 9 days in the presence of Vif-derived peptides and the amount of p24 CA antigen was determined. Fig. 5A shows that prolonged treatment of HIV-1_{IIIB} chronically infected Hut 78 cells with peptides 41, 6 and 7, but not with peptide 42 and 43, caused a reduction in the total amount of particulated and soluble p24 antigen present in the culture media.

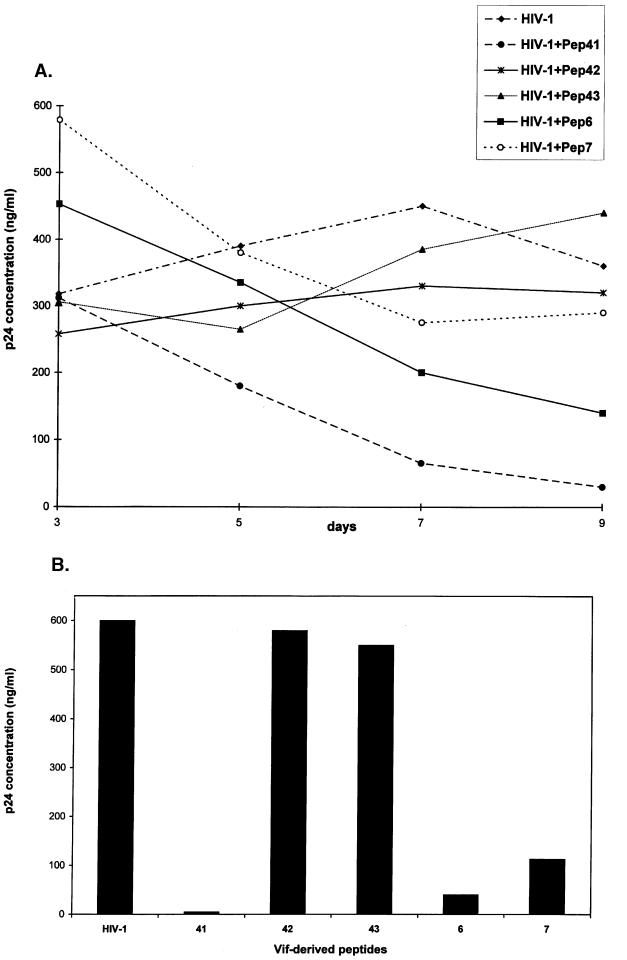

Fig. 5. Reduction of virus production followed by treatment with Vif derived peptides. A: Chronically infected Hut 78 cells (7.5×10^4 cells/well) 5 days post infection were cultured for 9 days in a 96 microwell plate in 200 μl of RPMI 1640 containing 60 μM of Vif-derived peptides. Half of the culture medium was replaced daily with fresh medium containing 30 μM of the tested peptide. Aliquots (100 μl) of cell-free medium removed at the indicated days were quantified for p24 CA antigen, using a Vironostika HIV-1 Antigen Microelisa System (Organon Teknika, USA). B: Newly infected cells were treated as described in A and p24 CA antigen was quantified after 9 days of treatment.

Similarly, treatment of newly infected Hut 78 cultures (at an HIV-1_{IIIB} MOI of 0.1) with peptides 41, 6, and 7 for 9 days reduced the release of CA antigen in the media, indicating less

Table 1
Infectivity of virus released from HIV-1-infected Hut 78 cells treated

Treatment	No. of LacZ-positive cells		Virus titer (IU/ml)[b]	Reduction of virus titer (%)
	undiluted[a]	1:10 dilution		
None (control)	1068	96	50 700	–
Peptide 41	137	6	4 925	91
Peptide 42	1120	81	48 250	5
Peptide 43	946	73	41 900	7
Peptide 6	63	2	2 075	96
Peptide 7	52	1	1 550	98
Ro31-8959	167	18	8 657	83

[a]20 μl of medium undiluted and 10-fold diluted harvested from HIV-1-infected Hut 78 cells treated with Vif-derived peptides (60 μM) or Ro31-8959 (100 nM) was used to infect HeLa CD4+ cells. The number of LacZ-positive cells is the average obtained from duplicate wells.
[b]IU = infectious units.
Representative data of three independent experiments are shown.

virus propagation in these cells (Fig. 5B). The inhibition of particle release is certainly not due to cytotoxicity induced by the Vif-derived peptides, because no difference in proliferation between cells treated with the peptides (up to 90 μM) and untreated controls cultured over a period of 9 days was found.

4. Discussion

Previously we demonstrated that Vif and N-Vif, but not C-Vif, inhibit the autoprocessing of truncated Gag-Pol polyproteins expressed in bacterial cells and inhibit the hydrolysis of synthetic peptide by purified PR in cell-free systems [29]. In an attempt to determine the minimal Vif sequence required to inhibit PR, we synthesized overlapping peptides derived from N-Vif and assessed them using in vitro and in vivo systems. The cell-free systems described here, namely inhibition and binding of PR by Vif-derived peptides, provide extreme flexibility to optimize reactions in order to observe, and potentially enhance, any subtle effects of Vif on protease. Peptides 4, 41, 6 and 7 derived from two stretches of N-Vif, encompassing residues Tyr-30–Val-65 and Asp-78–Val-98, inhibit PR activity and bind PR in cell-free systems. Peptides 41, 6, and 7 (peptide 4 is too large to penetrate the cells) suppress the autoprocessing of viral precursors expressed by vVK-1 and HIV-1 in human cells and inhibit the production of infectious virus. It is interesting to note that peptide 4, which tightly binds and inhibits PR, includes sequences that are conserved in HIV-2 and in other non-human lentiviruses [3], whereas domains including peptides 6 and 7 (amino acids 87–94) are antigenic [36] and relatively conserved among long-term AIDS survivors [37]. It is conceivable that although both regions are sequentially remote, they are spatially close and could, therefore, form an active site. Resolution of the three-dimensional structure of the Vif:PR complex and determination of the synergistic activity of the peptides might help to clarify the mode of action of Vif.

The activity of peptides 41, 6 and 7 differs from that of the PR inhibitor Ro31-8959. Ro31-8959 inhibits the processing of viral precursors in HIV-1-infected cells but allows the release of non-infectious particles containing unprocessed Gag polyprotein to the culture medium [35]. Inhibition of viral precursor processing was also observed in the same cells treated with the Vif-derived peptides, although $p55^{Gag}$ undergoes cleavage (Fig. 4). These cells released reduced amounts of p24 CA antigen to the medium, but unprocessed viral proteins were not detected in pellets prepared from these treated cells. The partial inhibition of viral precursor autoprocessing caused by peptides 41, 6 and 7 in the cells (Fig. 4A) may be sufficient to interfere with assembly and/or release of particles, as shown previously for other PR inhibitors [38–40]. Alternatively, these particles may be unstable and/or morphologically deformed, and therefore did not pellet through the sucrose cushions.

Our finding that Vif-derived peptides inhibit both the autoprocessing of Gag and Gag-Pol polyproteins in eukaryotic cells and the hydrolysis of synthetic peptides in vitro supports the supposition that Vif is responsible for the delay in PR activation, which lasts until assembly of the particles has taken place [29]. This delay is advantageous to both the virus and its host cell for the following reasons: (i) it ensures migration of viral structural components and catalytic enzymes to the site of virion assembly at an appropriate molar ratio; (ii) it hinders PR digestion of cellular proteins; and (iii) it prevents reverse transcriptase and integrase from acting on cellular RNA and causing unregulated gene amplification. Since PR is active as part of the Gag-Pol precursor [21–23], the delay should be operative at a post-translational stage. Moreover, it is most likely that the inhibition of PR is reversible, enabling the processing of viral precursors during particle assembly.

Regions in the C′-terminus of Vif are responsible for its binding to the inner surface of the outer cell membrane ([41], but see [42]). The same basic region of C-Vif is involved in binding two separate Gag domains, namely the NC and the MA/CA junction [16]. Our results indicate that peptides 41, 6 and 7, located in the N-Vif region, interact with PR. It is possible that all three binding sites play a pivotal role in the regulation of Gag and Gag-Pol processing. We suggest that regions of N-Vif inhibit the activation of PR when it is part of Gag-Pol. Upon arrival at the assembly site, the free C′ region of Vif interacts with the component(s) of the plasma membrane, allowing the initiation of processing. This is followed by the cleavage of Gag polyproteins, first in the SP-1/NC and later at the MA/CA junctions. As a result, the interaction between the Vif molecules and Gag is abolished, enabling the shift of Vif from the viral proteins to the outer cell membrane. Alternatively, the release of Vif molecules from all three Vif binding sites ensures appropriate processing. In the absence of the basic C′ region of Vif, the N-Vif-derived peptides remain bound to PR, and arrest the processing of the viral precursors. This model is consistent with the low levels of Vif present in virions [43] and with the finding that autoprocessing of viral precursors takes place at the plasma membrane where Vif is associated [38,44]. In transformed cells, where Vif is not essential for production of infectious virions (permissive cells), cellular protease inhibitor(s) might complement this Vif function.

This report, which describes a peptide mapping of N-Vif, clearly shows that three out of 11 peptides derived from N-Vif bind and inhibit HIV-1 PR in vitro, affect the processing of viral precursors in human cells and reduce the production of viral particles from infected cells. Thus, Vif-derived peptides provide an attractive potential therapeutic agent for inhibition of HIV-1 PR during infection in humans. The serious limitations of linear peptides as therapeutic agents are attributable to their susceptibility to proteolysis, poor bioavailability, unfavorable pharmacokinetics and rapid clearance. Conversion of the linear bioactive peptides described in this study into peptidomimetics (e.g. backbone cyclic peptides) is now under way in our laboratories.

Acknowledgements: This work was carried out in the Peter A. Krueger Laboratory with the generous support of Nancy and Lawrence Glick, and Pat and Marvin Weiss. We thank Roche Products Ltd. for Ro31-8959, Professor B. Moss (Laboratory of Viral Diseases, NIAID, NIH, Bethesda, MD, USA) for vVK-1, Dr. S.M. Nigida (Program Resources Inc., Frederick, MD, USA) for the monoclonal anti-CA, and Ms. Josefine Silfan for peptide synthesis. HIV-1 p24/25 Gag antiserum was obtained through the AIDS Reagent Program, Division of AIDS, NIAID, NIH.

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