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Human immunodeficiency virus type 1 (HIV-1) inhibition, DNA-binding, RNA-binding, and ribosome inactivation activities in the N-terminal segments of the plant anti-HIV protein GAP31

(peptide/AIDS/nucleic acid binding/antiviral protein)

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ABSTRACT **GAP31 (gelonium anti-HIV protein of 31 kDa) is an anti-HIV protein which we have identified and purified from a medicinal plant, *Gelonium multiflorum*. It is capable of inhibiting HIV-1 infection and replication. GAP31 also exhibits DNA topoisomerase inhibitor activity and RNA N-glycosidase activity. The ability of GAP31 to interrupt both DNA and RNA functions may be related to its multiple antiviral actions. To define the roles of these activities in the anti-HIV action of GAP31, a series of peptides corresponding to the N-terminal segment of GAP31 were synthesized and assayed for the aforementioned activities of the parent molecule. A 33-aa segment (KGATYITYVNFLNELRVKTKPEGNSH-GIPSLRK) designated as K10–K42 is the shortest peptide necessary and sufficient for HIV-1 inhibition, DNA and RNA binding, and ribosome inactivation. The peptides were 2–5 orders of magnitude less active than GAP31. Truncation of 19 aa from the C terminus of K10–K42 resulted in the loss of all of these activities. On the other hand, deletion of N-terminal residues to give E23–K42 did not alter ribosome-inactivation activity but eliminated the other activities. These findings permit identification of a 7-aa sequence, KGATYIT, at the N terminus of K10–K42 that is critical for DNA binding and RNA binding, whereas a 9-aa sequence, SHGIPSLRK, at the C terminus is important to ribosome inactivation. Both regions contribute to anti-HIV activity. Histidine at position 35 is critical for all of these activities. The disparity of sequence requirements for inhibition of HIV infection and replication and for ribosome-inactivation activity suggests that the anti-HIV activity of most ribosome-inactivating proteins may not be the result of N-glycosidase activity alone. Mapping the minimal domain of GAP31 offers insights into the rational design of molecular mimetics of anti-HIV drugs.**

Plants are a major natural source of drugs indispensable for health care. In search of antiviral agents, we have purified proteins of plant origins, MAP30, TAP29, GAP31, DAP30, and DAP32, which have been shown to inhibit both *de novo* HIV-1 infection and replication of the virus in already infected cells (1–3). In the effective dose range, little cytotoxicity to normal human cells and no toxicity to experimental animals were observed (1–4). N-terminal analyses of these proteins revealed 10–57% amino acid sequence homologies in the first 40–60 residues (3, 4). Furthermore, a homology of 48% was found between residues 1–40 of GAP31 and residues 659–698 of *Drosophila* DNA topoisomerase II. This led us to demonstrate a topoisomerase inhibitor activity in GAP31 (5). In addition, GAP31 and other anti-HIV proteins also exhibit ribosome-inactivating activity that hydrolyzes a glycosidic linkage between the ribose and adenine-4324 or guanine-4323 of 28S rRNA and subsequently inhibits eukaryotic protein synthesis (6–8). The dual action against both DNA and RNA demonstrated here for these series of proteins may be related to the inhibition of both infection and replication of HIV-1 by these compounds (4, 5). However, the mechanism underlying this unique dual function in the anti-HIV action of these plant proteins is still elusive. We first wanted to define the role of the N-terminal segment of GAP31 in the antiviral activities. In this report, we describe the synthesis of a series of peptide segments within aa 5–42 of the GAP31 sequence (3) and analyses of their DNA-binding, RNA-binding, ribosome-inactivation, and anti-HIV activities, including inhibition of syncytium formation, viral core protein p24 expression, and HIV reverse transcriptase (RT). Our results identify a 33-aa peptide, K10–K42, and a 20-aa peptide, E23–K42, as the smallest entities that exhibit anti-HIV activities and ribosome-inactivating activity, respectively.

MATERIALS AND METHODS

Chemical Synthesis and Purification of Peptides. Peptides were synthesized by the standard Merrifield solid-phase method on an Applied Biosystems model 430A peptide synthesizer and double coupling procedures using symmetric anhydrides or active esters of *t*-butyloxycarbonyl amino acids. Cleavage of the peptides from the resin and the protecting groups from the peptides was performed in either anhydrous HF or trifluoromethanesulfonic acid. Peptides were purified by Sephadex G-25 gel chromatography followed by reverse-phase HPLC on a Vydac C_4 column (packing pore size, 300 Å; particle diameter, 5 μM) with various eluting gradients composed of acetonitrile and 0.01 M trifluoroacetic acid. Purity of the peptides was evaluated by HPLC and amino acid composition.

Cells and Virus. The CEM-ss (syncytium sensitive, Leu-3 positive) cell line was used as the indicator cells for the microtiter syncytium-formation assay. The H9 and MT-4 T-lymphocyte lines were used for assays of p24 expression and virus-associated RT activity in suspension cultures.

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Abbreviations: HIV, human immunodeficiency virus; LTR, long terminal repeat; RT, reverse transcriptase; H35DNP, peptide Val^5–Lys^{42} (V5–K42) dinitrophenylated at His^{35}.

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Table 1. Sequences of GAP31-derived peptides and their alignments

Peptide	Sequence
V5–K42	VSFSTKGATYITYVNFLNELRVKTKPEGNSHGIPSLRK
$(CV5–K42)_2$	(VSFSTKGATYITYVNFLNELRVKTKPEGNSHGIPSLRK)$_2$
K10–N33	KGATYITYVNFLNELRVKTKPEGN
K10–K42	KGATYITYVNFLNELRVKTKPEGNSHGIPSLRK
K10–E23	KGATYITYVNFLNE
E23–K42	ELRVKTKPEGNSHGIPSLRK
Y17–K42	YVNFLNELRVKTKPEGNSHGIPSLRK
V5–E23	VSFSTKGATYITYVNFLNE
H35DNP	VSFSTKGATYITYVNFLNELRVKTKPEGNS**H**GIPSLRK

$(CV5–K42)_2$ is a dimer linked by disulfide bonds between N-terminal cysteines (these non-GAP31 cysteine residues are not shown). The dinitrophenylated histidine in H35DNP is shown in boldface type.

HIV-1 was prepared and stocked as described (9). The cell lines were cultured in RPMI 1640 medium containing penicillin (100 units/ml), streptomycin (100 μg/ml), and 10% heat-inactivated fetal bovine serum.

Anti-HIV Assays. Anti-HIV activity was measured by assay of microtiter syncytium formation in infectious cell centers (9), viral core protein p24 expression (10), and virus-associated RT activity (11).

Cytotoxicity and Cell Viability. The cytotoxicity of the peptides was measured by their effects on cellular syntheses of DNA and protein. The cells were incubated with 1 μCi (37 kBq) of [*methyl*-^{3}H]thymidine or L-[^{3}H]leucine 8 hr prior to harvest at day 4 postinfection. Cellular incorporation of labeled precursor into trichloroacetic acid-insoluble product was determined by scintillation counting. Cell viability was determined by trypan blue dye exclusion.

Ribosome-Inactivation Assay. Ribosome-inactivating activity was measured by *in vitro* translation in a rabbit reticulocyte lysate system (12) with globin message (3).

DNA- and RNA-Binding Assays. DNA binding and RNA binding were detected by electrophoretic mobility shift. Supercoiled DNA, mRNA, or rRNA (100–200 ng) was incubated in 10 μl with 20 μM peptide in 10 mM Tris·HCl, pH 7.8/50 mM NaCl/5 mM $MgCl_2$ at 30°C for 30 or 60 min. Samples (5 μl) of the reaction mixture with and without treatment with 0.5% SDS at 30°C for 10 min were loaded onto an agarose gel for electrophoresis. For DNA binding and rRNA binding, 1.0% and 1.6% agarose gels were used, respectively. For mRNA binding, 3.5% NuSieve (FMC) gels were used. Gels were stained with ethidium bromide (0.5 μg/ml) for 30 min and destained in distilled water prior to photodocumentation using a short-wavelength UV lamp.

RESULTS

Design and Synthesis of Peptides. When the N-terminal amino acid sequence of GAP31 was compared with sequences in the Genetics Computer Group (Madison, WI) data bank, homologies with the N-terminal sequence of various ribosome-inactivating proteins of plant origins were most profound. In addition, the N-terminal sequence of GAP31 aa 1–42 is also homologous to aa 659–691 and 678–720 of DNA topoisomerase II from *Drosophila melanogaster* and human, respectively. Since GAP31 exhibits both ribosome-inactivating and DNA topoisomerase inhibitor activities (3, 4) a segment, V5–K42, was first selected for chemical synthesis and biochemical assays. A cysteine residue was added on to make a disulfide-linked dimer $(CV5–K42)_2$ in an attempt to evaluate the effect of dimerization on the activities. His^{35} was dinitrophenylated at the imidazole moiety to examine the importance of His^{35} and the peptide was designated H35DNP. Intermediate peptides representing N-terminal portions (V5–E23 and K10–E23), C-terminal portions (E23–K42 and Y17–K42), and the middle section (K10–N33) were also synthesized to probe the contribution of each segment to the activities (Table 1).

Inhibition of Syncytium Formation. This assay is based on the interaction between fusigenic virus-infected cells expressing the HIV envelope gene products and uninfected adjacent cells bearing CD4 molecules and quantitates acute cell-free HIV-1 infection. V5–K42, $(CV5–K42)_2$, and K10–K42 displayed dose-dependent inhibition of HIV-1 infection and replication (Table 2) with an IC_{50} of ≈36 μM. The middle peptide, K10–N33, demonstrated partial anti-HIV activity. All other deletion derivatives and H35DNP exhibited no anti-HIV activity.

Inhibition of p24 Expression and HIV-Associated RT Activity. V5–K42, $(CV5–K42)_2$, and K10–K42 exhibited dose-dependent inhibition of p24 expression and RT activity (Fig. 1). The IC_{50} values for the two assays were similar, in the range 19–23 μM. The reduced p24 expression and HIV-associated RT activity were not due to cytotoxic or cytostatic effects, and no decrease in cellular DNA or protein synthesis was observed at these peptide concentrations. No inhibition

Table 2. Peptide inhibition of HIV-1 infection as measured by syncytium formation in the infectious-cell-center assay

Peptide, μM	% ICC (Vn/Vo) × 100						
	V5–K42	$(CV5–K42)_2$	K10–K42	K10–N33	K10–E23	Y17–K42	H35DNP
0	100	100	100	100	100	100	100
2	99	98	101	102	103	102	101
20	76	75	75	99	101	102	100
40	44	45	44	91	100	102	100
80	25	26	24	86	101	100	99
160	2	0	1	79	100	98	100
327	0	0	0	72	101	103	99
640	0	0	0	67	103	106	102

Values given in this table are averages of triplicates from two independent experiments. Triplicate wells of indicator cells containing peptides at each concentration without virus were also included for the determination of the cytotoxicity of these peptides. Infectious cell center (ICC) is expressed in terms of Vn/Vo, where Vn and Vo are average numbers of syncytia in peptide-treated and untreated samples, respectively. In the untreated control samples, the average number of syncytium-forming units per ICC per well was 96.

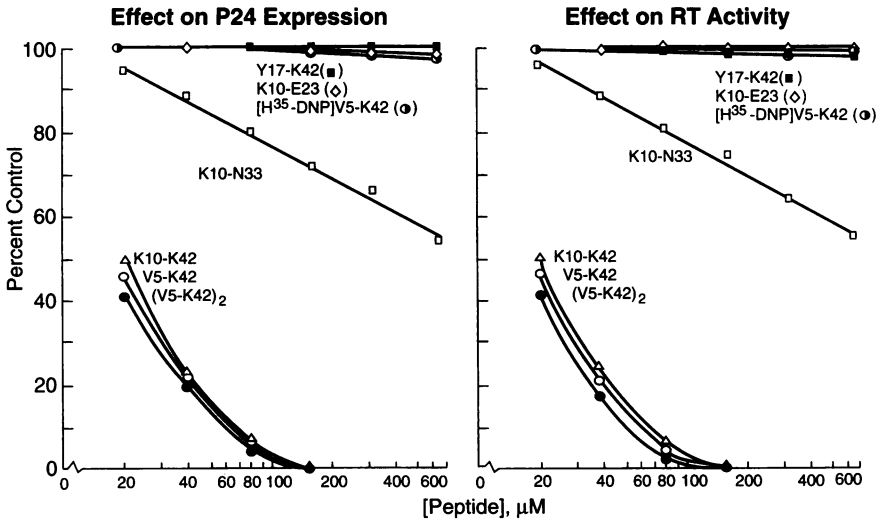

FIG. 1. Inhibition of replication of HIV-1 by K10–K42 and its analogues, as measured by viral core protein p24 expression (*Left*) and HIV-associated RT activity (*Right*).

of cellular incorporation of [^{3}H]thymidine or [^{3}H]leucine was observed even at 50 mM, or ≈2000 times the IC_{50} value. K10–N33 was less active in these assays, and other intermediate peptides and the H35DNP were inactive. Unlike 3′-azido-3′-deoxythymidine (AZT), K10–K42 and other peptides at 100 μM failed to inhibit the *in vitro* RT activity (data not shown).

Absence of Cytotoxicity of the Peptides. The cytotoxicity of K10–K42 and other peptides active in the anti-HIV assay was assessed by measuring their effects on cellular DNA and protein synthesis in uninfected H9 cells. A 1000-fold concentration range was tested (see Table 4). From 0.3 to 300 mM, these peptides caused no detectable effect on cellular incorporation of labeled thymidine or leucine, whereas p24 production and HIV RT activity were totally inhibited in HIV-infected cells.

Ribosome Inactivation. The ribosome-inactivation activity of the peptides was measured by *in vitro* translation in a rabbit reticulocyte lysate system using globin message (Table 3). At 20 μM, the average IC_{50} of HIV-1 inhibition of the three most active peptides (Fig. 1), complete inhibition of *in vitro* translation was observed. Y17–K42 and E23–K42, which were inactive in HIV inhibition, were as active as V5–K42, $(CV5–K42)_2$, and K10–K42 in ribosome inactivation. In contrast, V5–E23, K10–E23, and the H35DNP were inactive and K10–N33 was partially active. These results indicate that the C-terminal 20 aa of K10–K42 are critical to ribosome inactivation. Deletion of this region or modification at His^{35} results in the loss of ribosome-inactivation activity. The inhibition of *in vitro* translation seen with Y17–K42 and E23–K42 may indicate that the K10–N22 segment is not essential for ribosome inactivation but is essential for anti-HIV activity and for DNA and RNA binding, as will be shown below.

Table 3. Ribosome-inactivation activity of the peptides

Peptide	Gross cpm/μl	Net cpm/μl	% inhibition
Control (− mRNA)	3,062	—	0
Control (+ mRNA)	51,926	48,846	0
GAP31	2,614	—	100
V5–K42	2,616	—	100
$(CV5–K42)_2$	2,621	—	100
K10–K42	2,611	—	100
K10–N33	35,684	32,622	33
K10–E23	51,973	48,911	0
Y17–K42	2,599	—	100
E23–K42	3,102	40	99.9
H35DNP	44,635	41,573	15

Ribosome inactivation was measured by inhibition of *in vitro* translation in a rabbit reticulocyte lysate system using globin message. Inhibition of protein biosynthesis was determined by the incorporation of [^{3}H]leucine into trichloroacetic acid-insoluble material at 20 μM peptide, the average IC_{50} of HIV inhibition.

DNA Binding. HIV-1 long terminal repeat (LTR) supercoiled DNA was used as the substrate. V5–K42, $(CV5–K42)_2$, and K10–K42 were active in the binding of the DNA, as indicated by the retarded DNA–peptide complex (Fig. 2*A*, lanes 2, 3, and 5). The DNA–peptide complex was dissociated by 0.5% SDS (Fig. 2*B*, lanes 2, 3, and 5). Truncation of K10–K42 from either the N or C terminus resulted in significant for complete loss of DNA-binding activity (Fig. 2*A*, compare lane 6 with lanes 4 and 8). Modification of His^{35} also resulted in inactivation of DNA binding (Fig. 2*A*, lane 7). The characteristic of DNA binding for this series of peptides is different from the DNA topoisomerase inhibitor activity of GAP31. Treatment of supercoiled HIV LTR DNA (Fig. 2*C*, lane 1) with 0.02, 0.2, and 2 μg of GAP31 yielded relaxed and linear forms (lanes 2–4, respectively). This conversion was not reversed by SDS treatment (Fig. 2*C*, lanes 6–8). In addition to HIV LTR, the peptides are also capable of binding

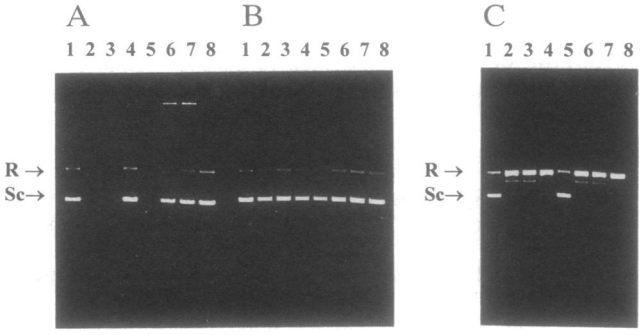

FIG. 2. DNA-binding activity of the peptides and GAP31. (*A*) Lane 1, HIV LTR supercoiled DNA substrate; lanes 2–8, DNA substrate incubated with V5–K42, $(CV5–K42)_2$, K10–E23, K10–K42, K10–N33, H35DNP, or Y17–K42, respectively. Positions of the relaxed (R) and supercoiled (Sc) DNA are indicated. (*B*) Reversibility of binding. Lanes 1–8, samples identical to corresponding lanes of *A*, but treated with 0.5% SDS. The anti-HIV peptides V5–K42, $(CV5–K42)_2$, and K10–K42 are capable of reversible DNA binding, whereas the inactive anti-HIV peptides K10–E23, H35DNP, and Y17–K42 are not. K10–N33 is partially active in DNA binding. (*C*) Relaxation of supercoiled HIV LTR DNA by GAP31 is not reversed by treatment with SDS. Lane 1, HIV LTR supercoiled DNA substrate; lanes 2–4, substrate treated with 0.02, 0.2, and 2 μg of GAP31, respectively; lanes 5–8, samples of lanes 1–4 treated with 0.5% SDS.

other viral DNAs, including ϕX174 replicative form, simian virus 40, and cytomegalovirus (data not shown).

RNA Binding. V5–K42, $(CV5–K42)_2$, and K10–K42 are capable of binding not only rRNAs (Fig. 3 *A* and *B*) but also mRNA (Fig. 3 *C* and *D*). The peptides are capable of binding both 28S and 18S rRNA but not 5S and 5.8S rRNAs or tRNAs. The structure–activity relationship for binding of the peptides to DNA completely parallels that for binding to RNA. No RNA binding was detected with Y17–K42 (Fig. 3*A*, lane 8) and E23–K42 (data not shown), even though they are fully active in ribosome inactivation. K10–N33 is partially active, and the shorter peptides are all inactive. These anti-HIV peptides are capable of binding globin messages (Fig. 3 *C* and *D*) as well as HIV-1 RNA. In a titration of rRNA binding with K10–K42 from 0–10 μM, dose-dependent binding was observed (Fig. 4*A*). At lower peptide concentrations, K10–K42 appears to bind 28S rRNA preferentially. At 20 μM peptide, virtually all of the 18 and 28S rRNAs were bound to K10–K42. The RNA–peptide complexes can be dissociated by SDS (Figs. 3*B*, 3*D*, and 4*B*). The DNA-binding and RNA-binding activities of the anti-HIV peptides are free of nuclease action, since intact DNA or RNAs and not their fragments were recovered upon dissociation of the binding complexes (Figs. 2–4).

Structure–Activity Relationship. The results of anti-HIV action, DNA binding, RNA binding, and ribosome inactivation are summarized in Table 4.

V5–K42 is active in HIV-1 inhibition as measured by syncytium formation, p24 expression, and HIV RT activity, as well as DNA binding, RNA binding, and ribosome inactivation. $(CV5–K42)_2$, the dimer of V5–K42, is as active as its monomer. K10–K42, an N-terminally truncated derivative of V5–K42, exhibits similar levels of activities of V5–K42 in all assay systems. Thus, K10–K42 is the smallest peptide capable of HIV-1 inhibition, DNA binding, RNA binding, and ribosome inactivation.

Truncation of 9 aa from the C terminus of K10–K42, yielding K10–N33, resulted in a significant loss of all activities. Neither the N-terminal portions, V5–E23 and K10–E23, nor the C-terminal segments, E23–K42 and Y17–K42, can inhibit HIV-1 or bind DNA and RNA. However, the two C-terminal segments possess significant ribosome-inactivating activity. Furthermore, these results also lead to the conclusion that the 7-aa segment KGATYIT at the N terminus of K10–K42 is critical for DNA binding and RNA binding, whereas the 9-aa segment SHGIPSLRK at the C terminus is important to ribosome inactivation. Both of these regions contribute to anti-HIV activity. The N-terminal sequence consisting of the first 9 aa in GAP31 is clearly not essential for any of the biological activities tested in the present study.

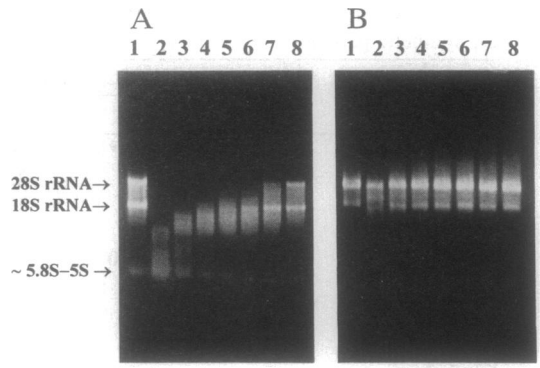

FIG. 4. Dose-dependent binding of rRNA by K10–K42. (*A*) Lane 1, control rRNA; lanes 2–8, control rRNA treated with K10–K42 at 10, 5, 2, 1, 0.5, 0.2, and 0.05 μM. (*B*) Corresponding samples treated with 0.5% SDS.

Dinitrophenylation of the imidazole moiety of the peptide V5–K42 resulted in complete inactivation of all the activities tested. K10–N33, which does not contain His^{35}, exhibits only minimal ribosome-inactivating activity. Clearly, a free imidazole ring is critical for full biological activities, including ribosome inactivation, HIV inhibition, and DNA and RNA binding.

DISCUSSION

The fragment K10–K42, which consists of 33 aa near the N terminus of GAP31 is the minimal essential fragment for HIV-1 inhibition. In addition, K10–K42 also possesses DNA-binding, RNA-binding, and ribosome-inactivation activities. The first 9 aa of GAP31 are thus not essential for HIV-1 inhibition, DNA binding, RNA binding, or ribosome inactivation. Our data indicate that anti-HIV activity and DNA- and RNA-binding activities require the next 33 aa, whereas ribosome-inactivating activity requires only the last 24 aa. This difference may reflect the separation of active sites responsible either for ribosome inactivation or for DNA and RNA binding. Consequently, HIV-1 inhibition is not merely

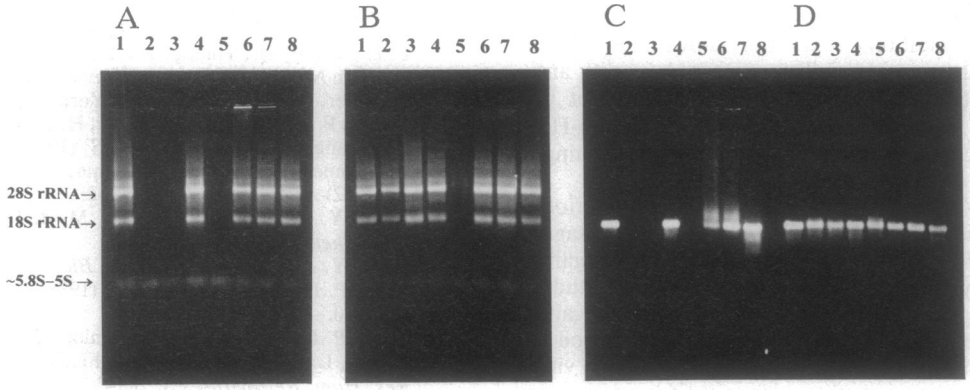

FIG. 3. RNA-binding activity of the peptides. (*A*) Binding of the peptides to rabbit reticulocyte rRNA. Positions of the 28S, 18S, and 5.8–5S rRNA are indicated. Lane 1, control rRNA; lanes 2–8, rRNA treated with V5–K42, $(CV5–K42)_2$, K10–E23, K10–K42, K10–N33, H35DNP, and Y17–K42, respectively. (*B*) Binding of the peptides to rRNA is reversed by SDS. Lanes 1–4, control rRNA and V5–K42-, $(CV5–K42)$-, and K10–K42-bound rRNA treated with 0.5% SDS; lane 5, same sample as in lane 4, but treated with 0.005% SDS, showing incomplete dissociation of the rRNA–peptide complex at low SDS concentration; lanes 6–8, samples identical to those in corresponding lanes of *A* but treated with 0.5% SDS. (*C*) mRNA binding of the peptides. Lane 1, control globin mRNA; lanes 2–8, globin mRNA treated with V5–K42, $(CV5–K42)_2$, K10–E23, K10–K42, K10–N33, H35DNP, and Y17–K42, respectively. (*D*) Dissociation of the mRNA–peptide complex by SDS. Lanes 1–8, samples identical to those in corresponding lanes of *C* but treated with 0.5% SDS.

Table 4. Comparison of anti-HIV, DNA-binding, RNA-binding, and ribosome-inactivating activity and cytotoxicity of the synthetic peptides

Peptides	Anti-HIV IC_{50}, μM			DNA binding	RNA binding	Ribosome inactivation	Cell toxicity
	Syncytia	p24	HIV RT				
GAP31	0.28*	0.23*	0.32*	+	+	+	>3200*
V5–K42	35	21	22	+	+	+	–
$(CV5–K42)_2$	36	19	20	+	+	+	–
K10–K42	36	22	23	+	+	+	–
K10–N33	>640	≈640	≈640	±	±	+	–
K10–E23	–	–	–	–	–	–	–
E23–K42	–	–	–	–	–	+	–
Y17–K42	–	–	–	–	–	+	–
V5–E23	–	–	–	–	–	–	–
H35DNP	–	–	–	–	–	–	–

Data on anti-HIV activities as measured by syncytium formation (Table 2), viral antigen p24 synthesis and HIV-1 RT (Fig. 1), DNA binding (Fig. 2), RNA binding (Figs. 3 and 4), and ribosome inactivation (Table 3) are from the table or figure indicated in parentheses. No cytotoxicity was detected at 300 mM peptide in uninfected H9 cells.
*Data obtained from ref. 4 (IC_{50}, nM).

due to ribosome inactivation. It also involves DNA binding and RNA binding.

In the assays for anti-HIV activity, K10–K42 gave IC_{50} values of 36, 22, and 23 μM for syncytium formation, p24 expression, and HIV RT activity respectively. Similar IC_{50} were obtained for the other two anti-HIV peptides, V5–K42 and $(CV5–K42)_2$. In all cases, the IC_{50} values for syncytium inhibition are slightly higher than those for p24 production and HIV RT activity. This variation may represent a difference in target cells, as well as in cell densities used in these assays. For GAP31 the corresponding IC_{50} values are 0.28–0.32 nM (3, 4). Thus, the synthetic peptides are about 5 orders of magnitude less active in anti-HIV activities and 4 orders of magnitude less active in ribosome inactivation. The reduced specific activity of peptide derivatives as compared with their parent molecule is not unexpected. This is often attributable to the stability and conformational constraints of the peptides. Furthermore, the lack of a DNA topoisomerase inhibitor domain may also contribute to the decrease in activity.

GAP31 has no detectable cytotoxicity (3). This may be due to its molecular size and lack of association with lectin-like carrier protein such as in the case of ricin and abrin. That it is toxic to the virus-infected cell may imply the change of cell surface properties upon viral invasion so as to allow the entrance of GAP31 and other proteins. This notion is supported by an immunofluorescence study using anti-GAP antibodies, which detected GAP31 in HIV-1-infected cells but not in uninfected cells after they were treated with GAP31 under the same conditions (data not shown). A similar experiment with the peptides will require high-avidity antibodies against the peptides; such antibodies are not yet available. In any event, the peptides active in anti-HIV activity lack detectable cytotoxicity at 30 mM, indicating a therapeutic index of at least 1000.

Since GAP31, V5–K42, and K10–K42 are multifunctional and appear to inhibit the HIV-1 life cycle at various stages, it seems reasonable to anticipate that similar activity against other viruses may exist. Indeed, our preliminary studies showed that both GAP31 and the peptides active against HIV-1 were active against cytomegalovirus as measured by direct fluorescent antibody test and against hepatitis B virus as assessed by measurement of its surface antigen expression in human hepatoma cell lines. Interestingly, either the protein or the peptides yielded the IC_{50} in the same order of magnitude among the three viruses tested.

The mechanism of antiviral action of GAP31 and its derived peptides remains to be defined. That GAP31 acts on virus-infected cells but not normal cells and that [^{35}S]methionine-labeled infected cells were found to yield greatly reduced levels of viral proteins indicate significant inhibition of viral protein synthesis by GAP31. Therefore, it seems unlikely that the agents affect viral budding in order to inhibit HIV infection/replication. Furthermore, the peptides' inability to inhibit *in vitro* RT activity implies that their anti-HIV action is different from that of 3′-azido-3′-deoxythymidine (AZT) (data not shown). Obviously, further investigation is warranted to elucidate the concerted effect of these anti-HIV peptides on DNA/RNA binding and ribosome inactivation in order to understand their mechanism of antiviral action.

Finally, it will be possible to carry out molecular modeling and dynamic analyses with the active anti-HIV peptides (13, 14). Defined anti-HIV derivatives, pseudopeptides, and peptidomimetics with desired pharmacokinetic properties and improved bioavailability may also be synthesized for clinical evaluations.

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