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Antiviral Activity of Influenza Virus M1 Zinc Finger Peptides

EZZELDIN H. NASSER,[1] AMRIT K. JUDD,[2] AGUSTIN SANCHEZ,[3] DIMITRIOS ANASTASIOU,[1] AND DORIS J. BUCHER[1]*

Department of Microbiology and Immunology, New York Medical College, Valhalla, New York 10595[1]; Bio-Organic Chemistry Laboratory, SRI International, Menlo Park, California 94025[2]; and Protein and Nucleic Acid Analytical Facility, Beckman Center, Stanford Medical School, Stanford, California 94305[3]

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Matrix protein (M1) of influenza virus inhibits its own polymerase; this suggested that a peptide segment of M1 with inhibitory properties could serve as an antiviral agent. A peptide synthesized to the Zn^{2+} finger region of the M1 sequence of influenza virus strain A/PR/8/34 centered around amino acids residues 148 to 166 was shown earlier to be 1,000-fold more effective as a polymerase inhibitor than M1. This peptide, designated peptide 6, represents a Zn^{2+} finger which includes a 7-residue "loop" and a 4-residue "tail" in addition to the 4 residues on either side of the loop involved in coordination of Zn^{2+}. We have now demonstrated antiviral activity for this peptide in microassays measuring inhibition of the viral cytopathic effect. When the peptide was introduced into tissue culture 5 min after viral challenge with A/PR/8/34, antiviral activity was seen at levels as low as 0.1 nM; on a molar basis, the peptide was shown to be 1,000- to 2,500-fold more effective than ribavirin or amantadine. Antiviral activity was seen with addition of the peptide up to 1 h after viral infection; however, little or no activity was seen at later times, suggesting that viral replication is inhibited at an early stage, possibly at the level of transcription. Reduction in the finger loop or tail length reduced antiviral activity; reduction in the number of residues involved in coordination of Zn^{2+} abolished antiviral activity. In addition to A/PR/8/34, peptide 6 was shown to have antiviral activity against other type A influenza viruses, including those representing H1N1, H2N2, and H3N2 subtypes. Antiviral activity against type B influenza viruses was also seen. A low level of activity against vesicular stomatitis virus was observed. Zn^{2+} finger peptides or analogs of Zn^{2+} finger peptides may provide a new class of antiviral agents effective against influenza virus and possibly other viruses.

The matrix protein (M1) encoded by RNA segment 7 is the most abundant protein found within the influenza virus virion and is believed to play a central role in virus assembly (2). M1 has been shown to inhibit influenza virus transcriptase (23–26); this effect can be reversed by monoclonal antibodies that recognize M1 (10). Peptides synthesized to two regions of M1 sequence (amino acid residues 90 to 109 and 129 to 164) were found to inhibit influenza virus transcriptase (23).

Wakefield and Brownlee demonstrated that M1 can bind RNA directly and proposed that this RNA-binding activity was mediated by residues 148 to 162, a region of M1 which resembles the zinc-binding motif found in RNA and DNA binding proteins (20, 21). Ye et al. showed that ^{32}P-labeled influenza virus RNA bound to both M1 and a chemically cleaved M1 peptide corresponding to residues 129 to 164 and suggested that the RNA binding activity may be due to the presence of a zinc finger motif in this region (23).

We demonstrated earlier that influenza virus transcriptase could be inhibited by a synthetic peptide based on the M1 sequence, residues 152 to 166, designated peptide 4 (12). Peptide 4 inhibited 38% of transcriptase activity at 0.05 μM and contained part of the zinc finger motif. By the addition of 4 residues at the amino terminus, another peptide, designated peptide 6 (residues 148 to 166), that contained a complete zinc finger was synthesized (Fig. 1). Peptide 6 was 1,000-fold more effective on a molar basis in transcriptase inhibition than was M1 (12).

The polymerase inhibitory properties of peptide 6 suggested that it could also have antiviral activity. This report describes studies of the antiviral activity of peptide 6 against influenza viruses.

MATERIALS AND METHODS

Viruses and cells. Influenza viruses used in this study included representatives of the H1N1 subtype (A/PR/8/34, A/USSR/90/77, and A/Texas/36/91), the H2N2 subtype (A/Japan/305/57), and the H3N2 subtype (A/Hong Kong/8/68, A/Port Chalmers/1/73, A/Bangkok/79, and A/Johannesburg/35/94). Type B influenza viruses included B/Lee/40 and B/Shanghai/4/94. Virus was produced by infecting 10-day-old chicken embryos and harvesting allantoic fluids 48 h after inoculation. MDCK (Madin Darby canine kidney) cells were cultured in minimal essential medium (MEM; GIBCO) supplemented with 10% fetal bovine serum. Influenza viruses were obtained from Edwin D. Kilbourne, New York Medical College. Vesicular somatitis virus (VSV) Indiana strain was obtained from Richard Peluso, Mount Sinai School of Medicine.

Peptide synthesis. The sequence of peptide 6 is from the sequence of M1 protein on the basis of A/PR/8/34 M1 sequence reported by Winter and Fields (22). The peptide and its analogs were synthesized by the solid-phase technique of Erickson and Merrifield (7) on a modified ABI/Perkin Elmer 431A peptide synthesizer with commercially available N^{a}-9-fluorenylmethyloxycarbonyl (Fmoc) amino acids attached to modified polystyrene resin and Fmoc-protected amino acids with the following side chain-protecting groups: tertiary butyl esters for aspartic acid and glutamic acid; tertiary butyl ether for serine and threonine; 2,2,5,7,8-pentamethyl chroman-6-sulfonyl for arginine; and Trityl for histidine, glutamine, and cysteine. After the completion of the synthesis, the peptide was cleaved from the resin with a cocktail containing 88% trifluoroacetic acid, 5% water, 5% phenol (liquid), and 2% diisopropylsilane. Crude peptides were purified with a Waters Delta Prep high-pressure liquid chromatography system on a preparative Millipore 25-mm by 10-cm C_{18} column radial compression module. A linear gradient extending from 10 to 26% acetonitrile (0.1% trifluoroacetic acid) in water was used. The purity of the peptides was at least 95%.

Assay of antiviral activity by inhibition of CPE. Microtiter plates were seeded with 10^4 MDCK cells per well. Monolayers were allowed to develop for 48 h in growth medium containing 10% fetal bovine serum at 37°C, resulting in 75% or greater confluency. The wells were washed with phosphate-buffered saline (PBS) and inoculated with 50 μl of seed virus (A/PR/8/34 or as indicated) diluted in PBS with 0.2% bovine serum albumin; final dilutions ranged from 10^4 to 10^{11}. After 5 min at room temperature, 50 μl of peptide, amantadine (Sigma), or

* Corresponding author. Mailing address: Department of Microbiology and Immunology, New York Medical College, Valhalla, NY 10595. Phone: (914) 993-4189. Fax: (914) 993-4176.

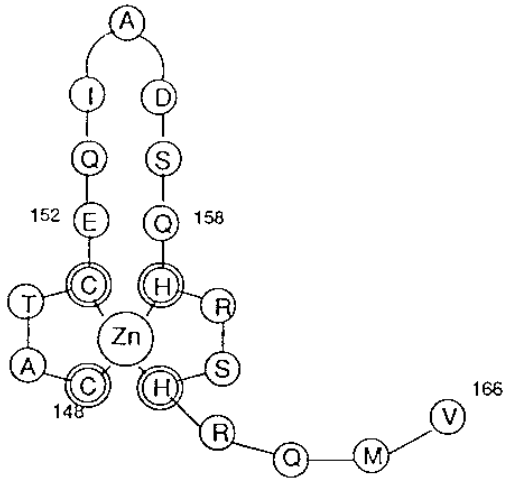

FIG. 1. Schematic folding scheme for a linear arrangement of peptide 6. Peptide 6 contains a cysteine- and histidine-rich sequence that is similar to the zinc finger sequence found in eukaryotic transcription factors. This structure may be important in inhibition of viral transcription, perhaps through direct interaction with viral RNA. The zinc finger domain is centered on a tetrahedral arrangement of the zinc ligand. The central residues 152 through 158 form a potential RNA-binding loop or finger.

ribavirin (ICN Pharmaceuticals) solution, depending on the experiment to be performed, was added at different concentrations in minimal essential medium (MEM) containing 0.2% bovine serum albumin, and the preparations were incubated for 30 min at 37°C. Then 100 μl of MEM with 2 μg of trypsin (VMF; Worthington Biochemical Corp.) per ml was added and the preparations were incubated at 37°C for another 48 h, after which the monolayers were stained with crystal violet (0.1% in 20% ethanol) and examined for cytopathic effect (CPE). One cytopathic unit (CPU) is defined as the highest dilution that results in total CPE and is equal to 1 to 2 PFU.

A time course experiment for antiviral activity was performed by adding 50 μl of peptide solution at different concentrations (1.0, 0.1, and 0.01 μM) in MEM at 0, 0.5, 1, 1.5, and 2 h to the microwell cell monolayers prepared as described above. At 30 min after the virus was added, 100 μl of MEM with 2 μg of trypsin per ml was added. After another 48 h, the monolayers were stained with crystal violet and examined for CPE.

The antiviral effect of preincubation with peptide 6 was examined by adding 50 μl of peptide solution at different concentrations in MEM to microwell cell monolayers prepared as described above and preincubating the mixtures for 24 h. The microwells were washed with PBS, incubated for an additional 0, 1, 2, 6, 12, or 24 h with MEM containing 0.2% bovine serum albumin without peptide, washed with PBS again, and inoculated with 50 μl of seed virus (A/PR/8/34) diluted in PBS containing 0.2% bovine serum albumin. The virus dilutions ranged from 10^4 to 10^{11}. The microwells were incubated for 30 min at 37°C, 100 μl of MEM with 2 μg of trypsin per ml was added, and the preparations were incubated for another 48 h. The monolayers were then stained with crystal violet and examined for CPE.

The antiviral activity of various analogs of peptide 6 was also examined by adding 50 μl of "tail" and "loop" analog peptides at different concentrations in MEM to microwell cell monolayers prepared as described above. At 30 min after the virus was added, 100 μl of MEM with 2 μg of trypsin per ml was added. After another 48 h, the monolayers were stained with crystal violet and examined for CPE.

The antiviral activities of various viruses were examined by adding 50 μl of seed virus diluted in PBS–0.2% bovine serum albumin to microwell cell monolayers; the dilutions ranged from 10^4 to 10^{11}. After 5 min at room temperature, 50 μl of peptide 6 solution at different concentrations in MEM with 0.2% bovine serum albumin was added to each well, and the preparations were incubated for 30 min at 37°C. Then 100 μl of MEM with 2 μg of trypsin per ml was added, and the preparations were incubated at 37°C for another 48 h, after which the monolayers were stained with crystal violet and examined for CPE.

RESULTS

Addition of peptide 6 to microwells protected the monolayers against a challenge of 100 CPU at 1 μM; antiviral activity was seen at levels as low as 0.1 nM with a challenge of 1 CPU (Fig. 2). The results are shown in tabular form in Table 1. Peptide 6 at 10 μM was toxic to MDCK cells.

When antiviral drugs were added to the microwell monolayers, protection was seen against a challenge of 1,000 CPU at 250 and 100 μM for amantadine and ribavirin, respectively, and against a challenge of 1 CPU at concentrations as low as 25 and 10 nM, respectively (Fig. 3). On a molar basis, peptide 6 was 2,500-fold more effective than amantadine and 1,000-fold more effective than ribavirin when tested with a challenge of 1 or 10 CPU (the concentration at midplateau shown in Fig. 3).

No antiviral activity was seen in the presence of irrelevant peptides including staphylococcal enterotoxin B (SEB 1-16 or SEB 148-162) or platelet-derived growth factor B (PDGF B 1-20). The absence of activity with irrelevant peptides shows that the antiviral effect is specific.

When a time course for the addition of peptide 6 was conducted, antiviral activity was seen when the peptide was added at 0, 0.5, and 1 h (Table 2), but little or no antiviral activity was seen at 1.5 h or later. This observation demonstrates that the peptide inhibits virus at an early stage in viral replication, perhaps through inhibition of the polymerase.

When cell monolayers were preincubated for 24 h with peptide 6 and the peptide was subsequently removed at various time points up to 24 h before viral challenge, full protection was seen at all time points (data not shown). This demonstrates that the peptide is retained by the cells and is capable of blocking viral replication for periods up to 24 h after peptide treatment.

To understand the important structural features of peptide 6 as they relate to antiviral activity, loop and tail analogs of peptide 6 were evaluated for the ability to inhibit viral CPE. Sequences of the various analogs are shown in Table 3. Reduction in finger loop length (residues 152 to 158) or tail length (residues 163 to 166) was accompanied by some degree of reduction in antiviral activity, as shown in Tables 4 and 5. Reduction of the number of residues involved in the coordination of Zn^{2+}, peptides 6Lx and 6Ly (Table 6), resulted in complete loss of antiviral activity. These results demonstrate that the lengths of the loop or tail regions are important but can be reduced with a substantial level of residual activity; however, the amino acid residues involved in Zn^{2+} coordination play a critical role in antiviral activity.

When various influenza virus strains (including both types A and B) were tested against peptide 6, various degrees of antiviral activity could be seen (Table 7). Some antiviral activity was also seen against a rhabdovirus, VSV. This demonstrates that peptide 6 has a broad spectrum of activity against both type A and B influenza viruses and may have some efficacy against viral groups other than orthomyxoviruses.

DISCUSSION

The zinc finger is a novel motif found in proteins which bind to DNA. It was originally discovered as a repeated sequence in *Xenopus* transcription factor TFIIIA (18). In this model, the cysteine and histidine pairs form a tetrahedral coordination site for a single zinc ion; amino acids between these coordination sites project out as fingers (18). Other DNA-binding proteins include yeast GAL4 transcriptional activator and mammalian steroid receptors; zinc fingers are formed by coordination with four cysteine residues (C-C; C-C zinc finger) (8).

Zinc finger regions of proteins appear to interact with DNA primarily through interactions in the major groove (19). Direct evidence for this interaction came from methylation interference experiments with TFIIIA from *Xenopus* oocytes and a 5S

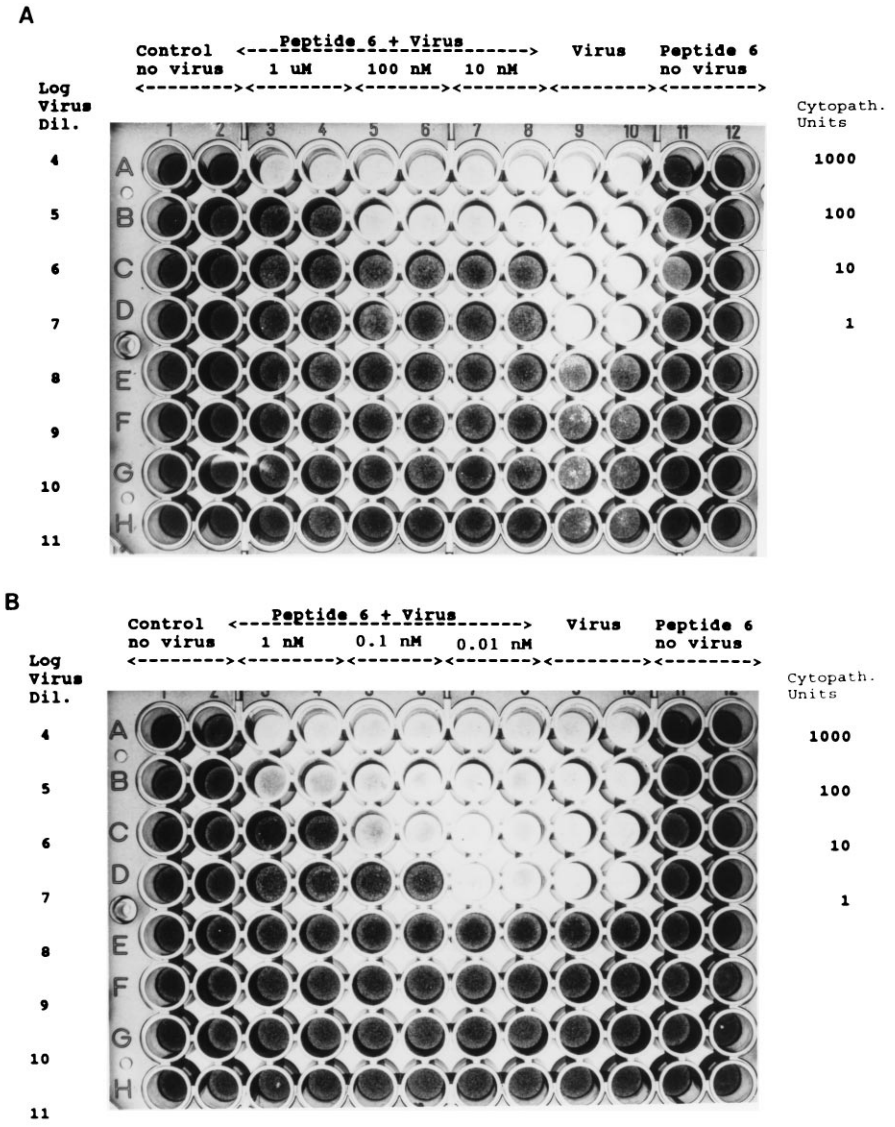

FIG. 2. Antiviral effect of peptide 6. Monolayers of MDCK cells on 96-well microwell plates were infected with various dilutions of virus (10^4 to 10^{11}) in columns 3 to 10 (rows A through H). No virus was added to columns 1, 2, 11, and 12. Infected cells were treated in duplicate with different concentrations of peptide 6 (1.0 μM to 0.01 nM); no peptide was added to columns 9 and 10 (virus control). (A) Peptide control (no virus) included columns 11A to 11H, 1.0 μM; 12A to 12D, 0.1 μM; and 12E to 12H, 0.01 μM. (B) Peptide control (no virus) included columns 11A to 11H, 1.0 nM; 12A to 12D, 0.1 nM; and 12E to 12H, 0.01 nM.

RNA gene; methylation at N-7 of guanine residues interfered with protein binding (19).

Subsequently, zinc fingers were found as a structural feature in a number of proteins that interact with RNA (5, 9, 14, 18).

TABLE 1. Antiviral activity of peptide 6

Concn of peptide 6 (nM)	Peptide protection against viral challenge (CPU) of:
1,000	100
100	10
10	10
1	10
0.1	1
0.01	0

Smaller zinc fingers consisting of 4 to 5 amino acids, "stubby fingers," have been found in some viruses, for example, the *gag* gene products of retroviruses and T4 phage gene *32* protein (13).

In studies of Moloney murine leukemia virus, Gorelick et al. used oligonucleotide-directed mutagenesis to generate mutations in the nucleocapsid protein-coding region containing a Zn^{2+} finger motif (9). They showed that the mutations destroyed the ability of viral protein to package viral RNA during virus assembly, suggesting that the zinc finger was involved in specific RNA recognition.

The Zn^{2+} finger motif is found in both human immunodeficiency virus types 1 and 2 (HIV-1 and -2). Mely et al. (17) showed the presence of stacking between the indole rings of tryptophan residues in positions 16 and 37 of the human HIV-1 nucleocapsid protein zinc finger and the bases of $tRNA^{Phe}$. Dannull et al. showed that the nucleocapsid (NC) protein of

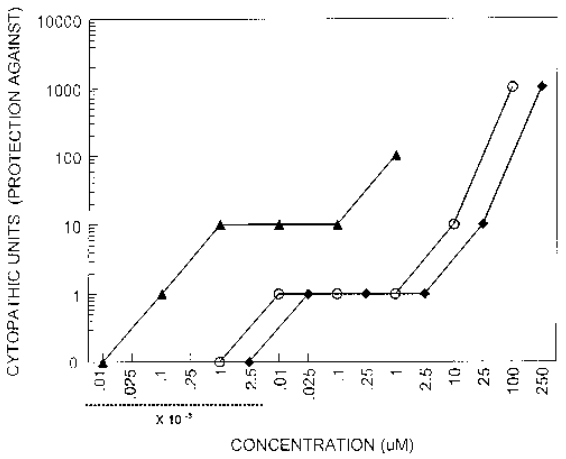

FIG. 3. Comparison of the antiviral activity of peptide 6 against amantadine and ribavirin. Monolayers of MDCK cells on 96-well microwell plates were infected with various dilutions of virus (10^4 to 10^{11}). Infected cells were treated in duplicate with peptide 6 (1.0 μM to 0.01 nM), amantadine (250 μM to 2.5 nM), and ribavirin (100 μM to 1 nM). Symbols: ▲, peptide 6; ◆, amantadine; ○, ribavirin.

TABLE 3. Peptide sequences of loop and tail analogs

Analog	Sequence	Comments[a]
Loop analogs		
6	CATCEQIADSQHRSHRQMV	7-aa loop
6a	CATCQIADSHRSHRQMV	5-aa loop
6b	CATCIADHRSHRQMV	3-aa loop
Tail analogs		
6	CATCEQIADSQHRSHRQMV	
6-1	CATCEQIADSQHRSHRQM	
6-2	CATCEQIADSQHRSHRQ	
6-3	CATCEQIADSQHRSHR	
6-4	CATCEQIADSQHRSH	
Tailless, loop, and Zn^{2+} coordination analogs		
6	CATCEQIADSQHRSHRQMV	
6L-2	CATCQIADSHRSH	
6L-4	CATCIADHRSH	
6Lx	CACIADHSH	
6Ly	CTCIADHRH	

[a] aa, amino acid.

HIV-1 (NCP7), which contains two zinc finger motifs, specifically binds to viral RNA containing the packaging site (5). In a Northwestern (RNA-protein) blot assay, Komatsu and Tozawa showed that the viral RNA-binding activity of HIV-2 nucleocapsid protein could be inhibited by a synthetic peptide containing the first zinc finger motif of HIV-2 (14).

The zinc finger found in M1 and synthesized as peptide 6 has the $C\text{-}X_2\text{-}C\text{-}X_n\text{-}H\text{-}X_2\text{-}H$ motif (X, amino acid residue; *n*, number of residues). This structure may inhibit viral transcription through direct interaction with viral RNA. The zinc finger domain is centered on a tetrahedral arrangement with a zinc ligand. The central residues 152 through 158 form a potential RNA-binding loop or finger. It was determined by electrospray ionization mass spectrometry that peptide 6 binds Zn^{2+} on a unimolar basis (11a).

Elster et al. demonstrated by atomic absorption spectroscopy that a 27-residue peptide containing the zinc finger region of influenza virus M1 binds zinc in a one-to-one complex (6). However, unlike our earlier results with peptide 6 (12) or those of Ye et al. (23) for M1 peptides containing the zinc finger region, these investigators found no transcriptase-inhibitory activity for their band. Furthermore, they found no RNA-binding activity for their peptide.

The arginine residues may also be involved in transcriptase inhibition and antiviral activity of peptide 6. Calnan et al. reported that arginine side chains on peptides may be commonly used to recognize specific RNA structures (4). Peptide 6 contains one arginine in the Zn^{2+} coordination region and a second arginine in the tail. Dannull et al. report the importance of flanking basic amino acids to the Zn^{2+} finger (5).

Peptide 6 was 1,000-fold more active as a polymerase inhibitor than was the original M1 (12). As an antiviral agent, peptide 6 was 2,500-fold more effective on a molar basis than amantadine and 1,000-fold more effective than ribavirin against a challenge of 1 to 10 CPU. Peptide 6 at high concentrations affected cell function; at 10 μM, peptide 6 was toxic to the cells and destroyed the cell monolayers.

Since little or no antiviral activity could be seen if the peptide was added later than 1 h after virus addition, the peptide clearly inhibits virus at an early stage in viral replication, presumably through inhibition of the polymerase. The similarity between the concentration of peptide 6 that results in antiviral activity (0.1 nM protects against a challenge of 1 CPU) and the concentration that causes inhibition of the viral polymerase (50% inhibitory concentration, 0.7 nM) further suggests that the mechanism of antiviral action is based on inhibition of the viral polymerase.

The CPE microassay is multicyclic. We believe that the reason why peptide added after 1.0 h offers little or no protection in the second cycle (and additional cycles) is that amplification of virus in the first cycle provides a viral challenge for the second cycle beyond the level of protection afforded by peptide 6. Peptide 6 was capable of protecting against an input of up to 100 CPU (approximately 100 to 200 PFU per well); it was not able to protect against an input of 1,000 CPU (a minimum of

TABLE 2. Antiviral activity of peptide 6 with respect to time of addition

Concn of peptide 6 (nM)	Peptide protection against viral challenge (CPU) at:				
	0 h[a]	0.5 h	1.0 h	1.5 h	2.0 h
1,000	100	100	100	1	0
100	10	10	10	0	0
10	10	10	10	0	0

[a] 0 h, peptide added 5 min after viral challenge.

TABLE 4. Antiviral activity of loop analogues of peptide 6

Concn of peptide 6 (nM)	Protection against viral challenge (CPU) by peptide:		
	6	6a	6b
1,000	100	100	10
100	10	10	10
10	10	1	1
1	10	1	1
0.1	1	1	1
0.01	0	0	0

TABLE 5. Antiviral activity of tail analogs of peptide 6

Concn of peptide 6 (nM)	Protection against viral challenge (CPU) by peptide:				
	6	6-1	6-2	6-3	6-4
1,000	100	10[a]	10	10	10
100	10	10	10	10	10
10	10	10	10	1	10
1	10	1	1	1	1
0.1	1	1	1	0	0
0.01	0	0	0	0	0

[a] There is partial protection at 100 CPU.

1,000 to 2,000 PFU per well would be expected following amplification in the first cycle).

The protection shown when cells were preincubated with peptide, which was removed before viral challenge, is significant. This demonstrates that the peptide can be retained by the cells for significant periods and provide protection against viral challenge, suggesting that the antiviral peptide could be used prophylactically against influenza viruses.

When loop and tail analogs of peptide 6 were tested for antiviral activity, reduction in finger loop length and in tail length reduced the antiviral activity to some extent. However, a reduction of the number of residues involved in Zn^{2+} coordination abolished antiviral activity. The number of residues in the Zn^{2+} coordination region for peptides 6Lx and 6Ly was reduced to 3 amino acids on either side of the loop; these peptides also have shorter loops (3-residue loops) than peptide 6. Peptide 6b, with a 3-residue loop and a 4-residue tail but a complete Zn^{2+} coordinating region, had substantial antiviral activity; peptide 6L-4 with a 3-residue loop, no tail, and complete Zn^{2+} coordinating region, retained some antiviral activity. Therefore, it appears that interference with Zn^{2+} coordination by shortening that region and presumable loss of Zn^{2+} binding results in the loss of antiviral activity. This demonstrates that this Zn^{2+}-binding region plays an important role in antiviral activity.

Peptide 6 had antiviral activity against a wide range of type A influenza viruses representing various subtypes as well as against two type B influenza viruses; a low level of activity against VSV was also seen. The M1 sequence representing peptide 6 is highly conserved among type A influenza viruses (11). Type A viruses used in our panel for which M1 sequences are available, in addition to A/PR/8/34, include A/USSR/90/77, A/Port Chalmers/1/73, and A/Bangkok/1/79 (as summarized by Ito et al. [11]). Among these, only A/Bangkok/1/79 M1 differs in sequence; the histidine at position 162 has been replaced with a leucine, which should interfere with Zn^{2+} coordination. The lowest antiviral activity of peptide 6 was seen against this type A virus. However, substantial antiviral activity was seen against type B influenza viruses, especially B/Lee/40, which preserves the Zn^{2+} finger motif but has only 42% homology to A/PR/8/34 in this region of M1 (15).

TABLE 6. Antiviral activity of tailless, loop, and Zn^{2+} coordination analogs of peptide 6

Concn of peptide 6 (nM)	Protection against viral challenge (CPU) by peptide:				
	6	6L-2	6L-4	6Lx	6Ly
1,000	100	10	1	0	0
100	10	1	1	0	0
10	10	1	0	0	0

TABLE 7. Antiviral activity of peptide 6 on influenza A and B viruses and VSV

Virus strain[a]	Protection against viral challenge (CPU) by concn (nM):		
	1,000	100	10
A/PR/8/34 (H1N1)	100	10	10
A/USSR/90/77 (H1N1)	10	10	1
A/Texas/36/91 (H1N1)	10	1	1
A/Japan/305/57 (H2N2)	10	10	1
A/Hong Kong/8/68 (H3N2)	10	10	1
A/Port Chalmers/1/73 (H3N2)	10	10	1
A/Bangkok/1/79 (H3N2)	1	1	0
A/Johannesburg/35/94 (H3N2)	10	10	1
B/Lee/40	10	10	1
B/Shanghai/4/94	1	1	1
VSV Indiana	1	1	1

[a] All viruses are influenza virus strains except for VSV Indiana.

With respect to the low level of antiviral activity seen against VSV, Ye et al. showed that influenza virus M1 could inhibit VSV transcription (25). However, their results suggest that inhibition is not related to the Zn^{2+} finger region of M1. Furthermore, no Zn^{2+} finger motif is found in the VSV matrix protein (25).

We assume that peptide 6 must enter the cell to produce antiviral activity. Antiviral activity was seen 24 h after treatment of cells, removal of peptide 6 from the medium, and exposure to virus; evidently, the cells retain the peptide. At 19 residues, the peptide would appear to be too large for entry into the cell. It is possible that with coordination of Zn^{2+}, the peptide can assume a compact size which more readily enters the cell than a peptide which is 19 residues and has an extended conformation.

Influenza virus transcription takes place in the nucleus; Bucher et al. demonstrated that M1 localizes to the nucleus and nucleolus (3). It may be necessary for the peptide to enter the nucleus to exert its antiviral effect; alternatively, it may be sufficient for the peptide to enter the cytoplasm. The peptide may bind to the RNA of the nucleocapsid while the nucleocapsid is in the cytoplasm and thus block RNA polymerase activity after transport of the nucleocapsid to the nucleus, or the peptide may enter the nucleus and then bind to the RNA and block RNA polymerase activity. It is noteworthy that the RNA of influenza virus in the nucleocapsid is exposed; nucleoprotein does not protect the RNA against the activity of RNase (23). Therefore, the RNA may be accessible for binding of the peptide.

The only antiviral agents currently available for clinical use against influenza virus are amantadine and its close relative rimantadine (1). Although these drugs can be quite effective against type A influenza virus (approximately 70 to 90% effective in preventing illnesses caused by type A influenza viruses), they are not effective against type B influenza virus (1). The drugs also have significant side effects including nausea, dizziness, insomnia, nervousness, and impaired concentration (1). Resistance to these compounds develops rapidly, and transmission of resistant viruses also may occur (16).

New antiviral agents against influenza viruses are certainly needed. In vivo studies have been performed with peptide 6,

using a mouse influenza model; when administered intranasally, peptide 6 was found to be as active as ribavirin against A/PR/8/34 (H1N1) and more active than ribavirin against A/Victoria/3/75 (H3N2) (12a). Peptide 6 may provide a new approach to the design of antiviral agents effective against influenza virus and possibly other viruses.

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