ACS Infectious Diseases

Letter

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Influenza Viral Hemagglutinin Peptide Inhibits Influenza Viral Entry by Shielding the Host Receptor

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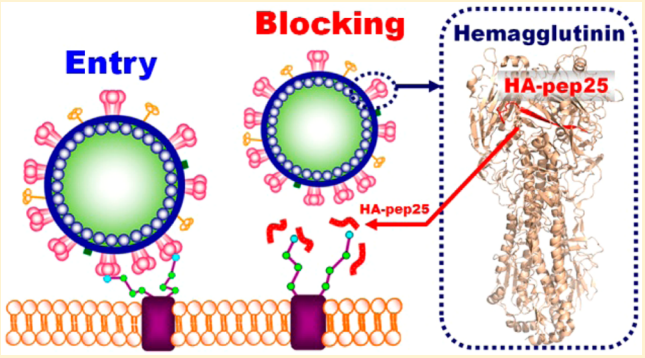
Supporting Information

ABSTRACT: Influenza viral infection of the host begins by the attachment of viral hemagglutinin to a cell surface receptor. In the current study, a hemagglutinin fragment peptide library was screened using an H5N1 recombinant pseudotyped viral system. One peptide, designated HA-pep25, showed effective antiviral activity against both human and avian influenza viral strains (IC_{50} = 12.0−51.0 μM). A mechanistic study demonstrated direct binding between HA-pep25 and sialyllactose, which mimics the host receptor for the influenza virus. This binding was independent of the presence of sialic acid on the cell membrane. By generating alanine substitutions in HA-pep25, eight residues were identified as essential for the peptide's anti-influenza activity. HA-pep25 derived from hemagglutinin blocked influenza viral entry by shielding the host receptor on the cell membrane. This peptide might be a candidate drug for influenza virus entry inhibition and may be combined with other antivirals targeting different steps of the influenza viral life cycle.



KEYWORDS: *influenza, hemagglutinin, peptide, entry inhibitor, host*

Influenza viruses are enveloped, segmented, negative-sense, single-stranded RNA viruses that cause approximately 1 billion cases of infection, resulting in approximately 0.5 million deaths, each year.[1] There are three types of influenza virus: A, B, and C. Influenza A virus is the most epidemic type, and it has numerous strains. The two viral envelope glycoproteins, hemagglutinin (HA) and neuraminidase (NA), determine the viral subtype. In total, 18 HA subtypes and 11 NA subtypes have been identified.[2] In theory, these subtypes can be randomly assembled into new strains. Although the known pandemic influenza strains affecting humans are H1N1, H2N2, and H3N2 viruses,[3] new strains continually appear because of the high mutability and recombinant ability of the influenza virus. The locations and types of new strains that appear cannot currently be predicted.

There are currently two classes of approved antiflu drugs,[4] M2 channel inhibitors and NA inhibitors. The M2 inhibitors (amantadine and rimantadine) influence the function of the viral ion channel M2 protein. The NA inhibitors (oseltamivir, zanamivir, peramivir, and laninamivir) interfere with the release of progeny viruses through competitive combination with viral NA. However, because most currently circulating influenza viruses are resistant to M2 inhibitors, amantadine and rimantadine are no longer recommended as common clinical anti-influenza treatments.[5] Furthermore, mutant viruses resistant to NA inhibitors have also been detected in humans.[6,7] The fact that these drugs have a single target limits anti-influenza countermeasures and creates intense selection pressure in circulating viruses; thus, new drugs targeting various steps of the influenza viral life cycle are urgently needed.

Influenza viral entry is an attractive target for anti-influenza drugs. However, no entry inhibitor is clinically available at the present time. Influenza viral HA is a key component for viral entry. HA is composed of two subunits. HA1 is responsible for recognizing and binding with the host receptor, and HA2 mediates the fusion of the viral and cellular membranes. The influenza virus receptor is thought to be the sialyloligosaccharides, which decorated at the terminal ends of cell surface glycoconjugates. The binding of the influenza virus to the receptor is species specific. For example, avian viruses prefer to bind sialic acid through α2,3-linkages to galactose, whereas viruses infecting humans prefer to bind α2,6-linked sialic acid.[8] However, this difference is insufficient for preventing cross-species viral transmission. As long as the virulent strains overcome species boundaries, pandemics will break out, such as the highly pathogenic avian influenza A (H5N1) epidemics in 1997[9] and 2003−2005,[10] the swine flu H1N1 epidemic in 2009,[11] and the avian flu H7N9 epidemic in 2013.[12] Although the influenza vaccine is always effective for pandemics' control, the 6 month preparation time prevents a response for the first wave of infections of new pandemic influenza viruses.[13] Therefore,

Received: November 18, 2015
Published: January 29, 2016

DOI: 10.1021/acsinfecdis.5b00139
ACS Infect. Dis. 2016, 2, 187−193

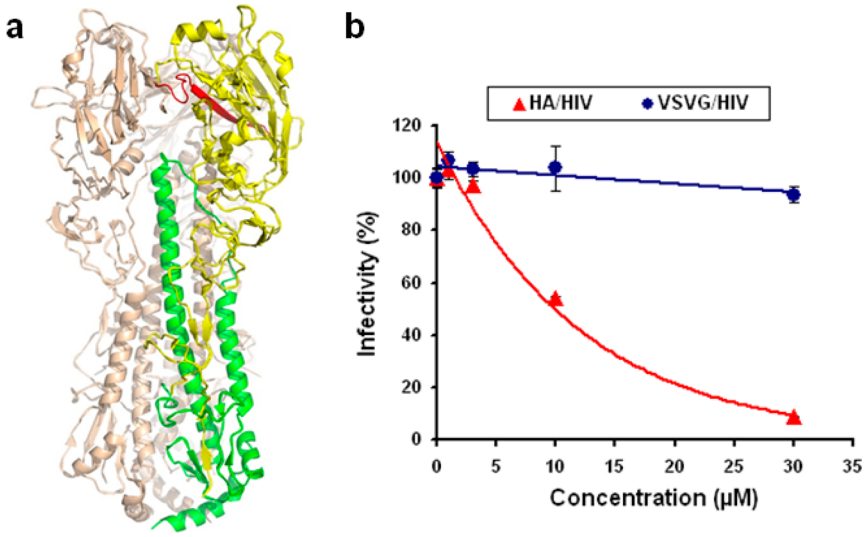


Figure 1. (a) Structure of HA derived from the highly pathogenic H5N1 strain (A/Vietnam/1203/2004) shown as a trimer (PDB 2FK0). The HA1 and HA2 subunits are colored yellow and green, respectively. HA-pep25 (N-SKVNGQSGRMEFFWTILK-C) is colored red. (b) Identification of the activity and specificity of HA-pep25 using HA/HIV and VSVG/HIV pseudovirions.

besides quarantine measures, drugs serve as a first-line emergency defense. The discovery of new drugs that can function to inhibit infections caused by both human and avian viruses is important.

There are two major strategies for the discovery of influenza entry inhibitors. The first strategy is targeting the virus, such as receptor-mimic compounds or peptides targeting HA1,[14−16] compounds or peptides blocking HA2-mediated membrane fusion,[17−19] monoclonal antibodies to HA,[20,21] and natural products targeting HA.[22] However, most of the above inhibitors are specific for certain strains, which limits their broad application. The second strategy is aimed at the host, mostly through targeting receptors or cofactors, such as the recombinant fusion protein DAS181, which has sialidase catalytic activity,[23] and sialylgalactose-binding peptide inhibitors selected from a phage library.[24] Although influenza receptors vary among different species, the key elements involved in viral binding are fixed, including the terminal sialic acids and oligosaccharides on the cell surface. Thus, inhibitors targeting the host cell could be more amenable for broad application, and viral resistance to these inhibitors would not develop as quickly as that against inhibitors that directly target the virus. Compared to random phage library screening, an HA-derived peptide library is also an attractive idea. HA2 subunit-derived fusion inhibitors targeting the structural changes of HA2 have been previously reported;[19] however, to the best of our knowledge, host-targeting peptide inhibitors derived from the HA1 subunit have not been previously reported.

In this study, an HA fragment peptide library was synthesized and screened for identification of effective peptides targeting host cells. HA-pep25, an 18-mer peptide corresponding to residues 221−238 of HA1, showed inhibitory activities against all four influenza viruses tested, including human H1N1, human H5N1, avian H5N1, and avian H7N9 viruses. Mechanistically, HA-pep25 blocked the HA−cell interactions by binding to the influenza host cell receptor sialyllactose, and this binding was independent of the presence of sialic acid on the cell membrane. We also found that both the length of the peptide and eight key residues within the peptide were essential for HA-pep25's activity. As it can inhibit influenza virus entry into the cells, it is our hope that HA-pep25 could be used in the future as a peptide spray that is inhaled into the respiratory tract (similar to the route of administration for zanamivir and laninamivir). Alternatively, HA-pep25 could be used in combination with current antivirals.

RESULTS AND DISCUSSION

The first step of influenza viral entry into a host cell is mediated by interactions between HA on the viral surface and cell surface receptor on host cells. Protein fragments derived from HA receptor binding domain (RBD) showed an anti-influenza effect, which has been reported,[25] but the immunogenicity and high costs associated with their production limit their use in the clinic. However, peptides are druggable.[26] In this study, an HA fragment peptide library based on influenza viral HA (gene accession no. AY818135) derived from the highly pathogenic H5N1 virus (A/Vietnam/1203/2004, VN/04) was constructed (Figure S1; Table S1). The library contained 59 peptides that were 18 amino acids in length with 9 residues overlapping with the adjacent peptides. All peptides were screened at a final concentration of 100 μM using a human immunodeficiency virus (HIV)-based pseudotyped system.[27] In this system, recombinant HA/HIV pseudovirions contained the influenza viral HA (VN/04) envelope surrounding an env-deficient HIV core, which contained an inserted luciferase reporter gene. In infected cells, the expressed amount of luciferase reflects the level of viral entry. Recombinant vesicular stomatitis pseudovirions (VSVG/HIV) were utilized as a specific control model to exclude the possibility that the inhibitors were targeting HIV replication. As shown in Figure 1, HA-pep25 (N-SKVNGQSGRMEFFWTILK-C), the sequence of which is derived from residues 221−238 of HA1, showed a dose-dependent inhibitory effect on recombinant H5N1 (VN/04) influenza viral entry with an IC_{50} of 12.0 μM (Figure 1). We further examined the effects of HA-pep25 on other types of influenza virus. HA-pep25 effectively inhibited human H1N1 (A/PuertoRico/8/1934) viral infection with an IC_{50} of 34.0 μM, and this inhibitory activity was stronger than that produced by our positive control drug we tested simultaneously (ribavirin, a replication inhibitor,[28] IC_{50} = 65.0 μM). Meanwhile, HA-pep25 effectively inhibited two other influenza pseudoviruses, the avian H5N1 and avian H7N9 viruses

DOI: 10.1021/acsinfecdis.5b00139
ACS Infect. Dis. 2016, 2, 187−193

(Table 1). According to the HA phylogenetic analysis, the H1 and H5 subtypes belong to influenza A group I, whereas the H7 subtype belongs to group II.[29] Sequence alignment of the four HAs used in this study showed low homology (Figure S2). This indicates that HA-pep25 likely has a broad range of anti-influenza effects.

Table 1. Effects of HA-pep25 Peptide on Different Strains of Influenza A Virus

HA serotype	strain	species	IC_{50} (μM)
H1	A/PuertoRico/8/1934	human	34.0 ± 11.5
H5	A/Vietnam/1203/2004[a]	human	12.0 ± 2.1
	A/Goose/Qinghai/59/2005[a]	avian	34.8 ± 1.5
H7	A/Duck/Anhui/SC702/2013[a]	avian	51.0 ± 0.6

[a]HA/HIV pseudoviruses were used for these assays.

To determine the mechanism, a biotinylated HA-pep25 was synthesized. This biotin−HA-pep25 bound to Madin−Darby canine kidney (MDCK) cells in a dose-dependent manner. An invalid peptide, biotin−HA-pep3 (biotin−IMEKNVTVTHAQ-DILEKK) was used as a negative control and showed no binding to MDCK cells (Figure 2a). Immunohistochemical staining to detect HA-pep25's interactions with mouse lung tissue samples revealed similar results (Figure 2b). These results indicate that HA-pep25 targeted the host. Because sialyloligosaccharides are the influenza virus receptors on host cells, we assessed the interaction of HA-pep25 and sialyllactose using surface plasmon resonance (SPR) analysis. As shown in Figures 2c and S6, HA-pep25 bound sialyllactose in a dose-dependent manner, which suggests that HA-pep25 can target influenza virus receptor on host cells. Interestingly, further mechanism explorations reveal that HA-pep25 remained bound to host cells even when the cell surface sialic acids were removed via neuraminidase treatment (Figure 2d). Additionally, the pre-incubation of sialic acid

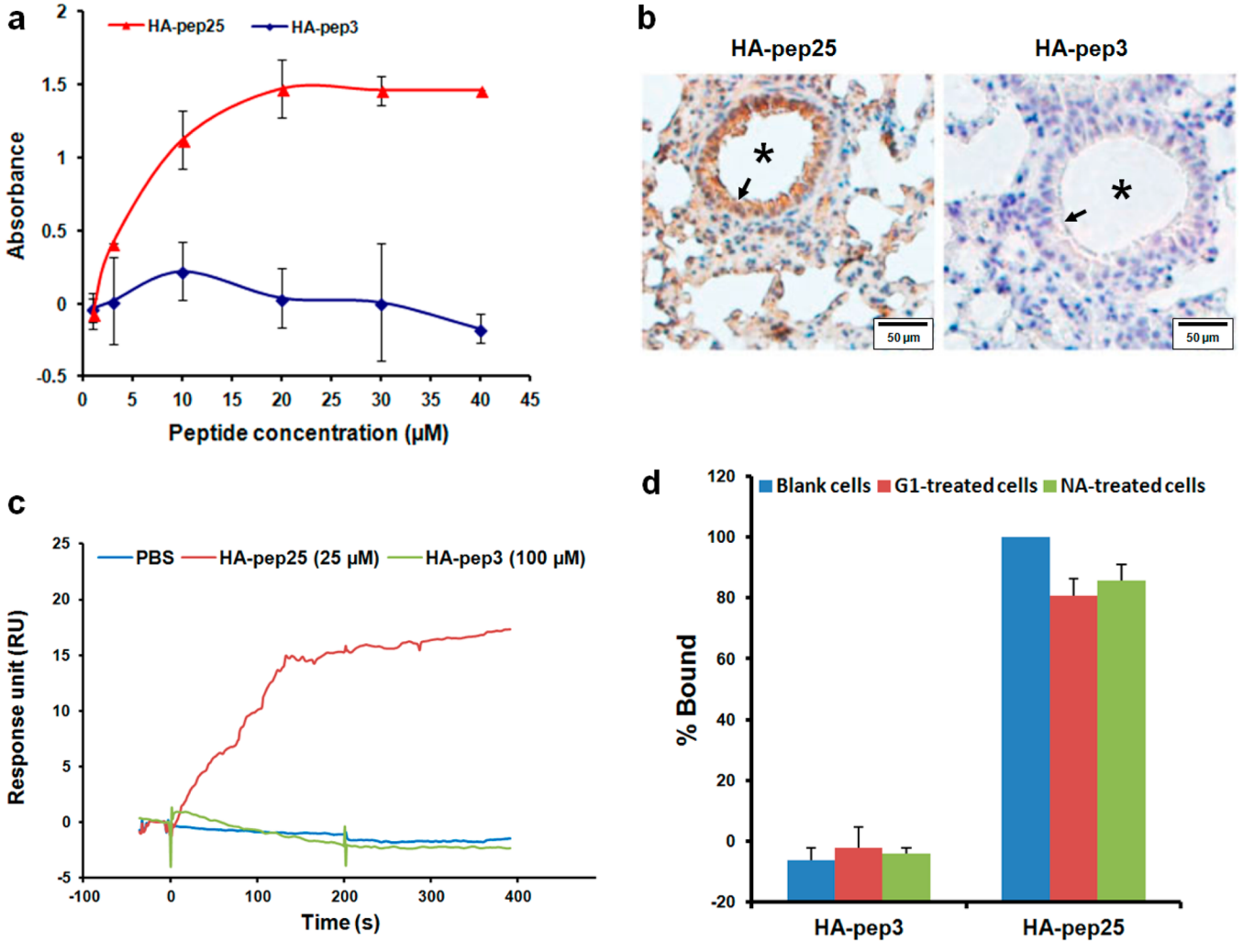


Figure 2. (a) HA-pep25 bound to MDCK cells in a dose-dependent manner. Biotinylated HA-pep25 or biotinylated HA-pep3 was incubated with MDCK cells. HA-pep25 demonstrated significant binding to the cells, whereas HA-pep3 showed no obvious binding. (b) HA-pep25 directly bound to lung/tracheal cells. Biotinylated HA-pep25 was incubated with mouse lung tissue (shown in brown around the lung/tracheal cells). The asterisk indicates the lumen of the trachea, and the arrow indicates the tracheal cell surface. (c) HA-pep25 directly bound to sialyllactose. Binding was measured using surface plasmon resonance analysis. HA-pep25 bound to sialyllactose at 25 μM, whereas HA-pep3 could not bind at 100 μM. (d) HA-pep25 could bind to sialic acid-free MDCK cells. To test this, MDCK cells were treated with α2−3,6,8 neuraminidase to remove the terminal α2,3-, α2,6-, and α2,8-linked sialic acids from glycoconjugates on cell surface. G1 buffer treated MDCK cells were used as a reagent control (Figure S5). For the binding assay, the peptides were used at a concentration of 10 μM.

DOI: 10.1021/acsinfecdis.5b00139
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a

Peptide	Sequence
HA-pep25	SKVNGQSGRMEFFWTILK
Short peptide	
sh1	SKVNGQ
sh2	SKVNGQS
sh3	SKVNGQSGR
sh4	SKVNGQSGRMEF
sh5	SKVNGQSGRMEFFWT
Site-mutated peptide	
No. /Residue	
m1 /Ser_1	AKVNGQSGRMEFFWTILK
m2 /Arg_2	SAVNGQSGRMEFFWTILK
m3 /Val_3	SKANGQSGRMEFFWTILK
m4 /Asn_4	SKVAGQSGRMEFFWTILK
m5 /Gln_6	SKVNGASGRMEFFWTILK
m6 /Ser_7	SKVNGQAGRMEFFWTILK
m7 /Arg_9	SKVNGQSGAMEFFWTILK
m8 /Met_{10}	SKVNGQSGRAEFFWTILK
m9 /Glu_{11}	SKVNGQSGRMAFFWTILK
m10 /Phe_{12}	SKVNGQSGRMEAFWTILK
m11 /Phe_{13}	SKVNGQSGRMEFAWTILK
m12 /Trp_{14}	SKVNGQSGRMEFFATILK
m13 /Thr_{15}	SKVNGQSGRMEFFWAILK
m14 /Ile_{16}	SKVNGQSGRMEFFWTALK
m15 /Leu_{17}	SKVNGQSGRMEFFWTIAK
m16 /Lys_{18}	SKVNGQSGRMEFFWTILA

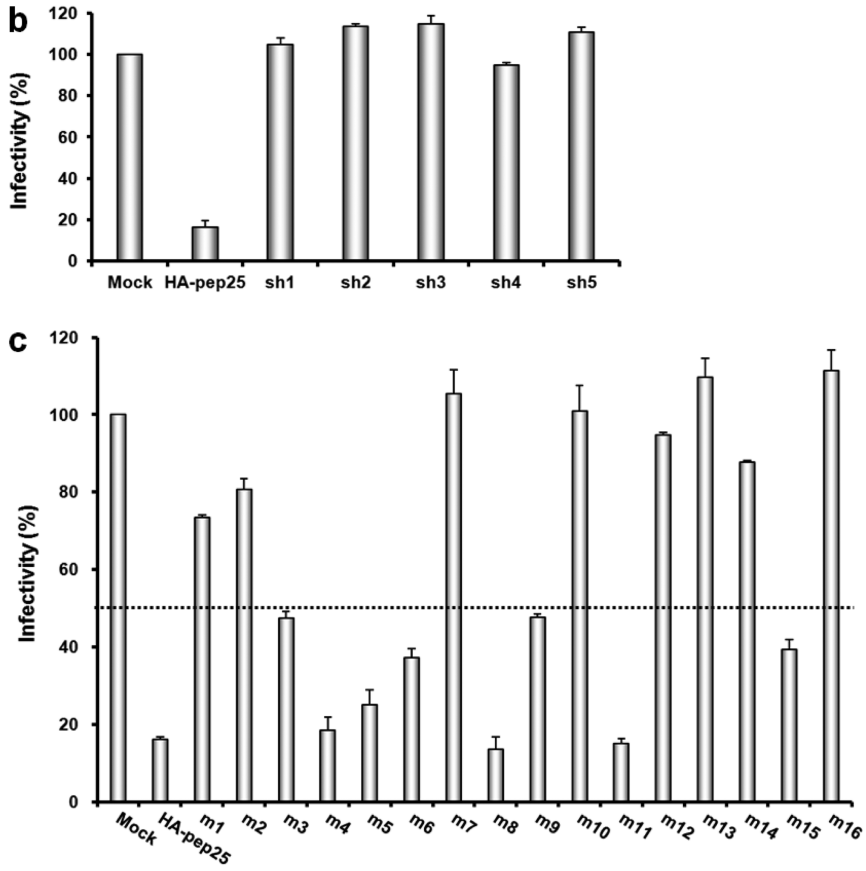

Figure 3. (a) Sequences of derivative peptides based on HA-pep25. (b, c) Effects of shortened peptides (b) or site-mutated peptides (c) on HA/HIV viral entry. All peptides were tested at a concentration of 30 μM. The mock was HA/HIV viral infection without any peptide.

analogue (2-*O*-methyl-α-D-*N*-acetylneuraminic acid, 20 mM) with HA-pep25 (10 μM) could not block the binding of HA-pep25 to host cells (Figure S3). Thus, the terminal sialic acids that determine the species specificity of influenza virus do not serve as its target, and this may be the reason for the ability of HA-pep25 to inhibit both human and avian influenza viral infections. The abilities of D-lactose (β-D-Gal-(1−4)-D-Glc) and α-lactose (β-D-Gal-(1−4)-α-D-Glc) to interfere with HA-pep25 binding to MDCK cells were also evaluated, and neither lactose affected this interaction (Figure S3). Collectively, on the basis of all of the above data, we have concluded that the inhibitory activity of HA-pep25 against influenza viral entry arises from the ability of the peptide to bind to the oligosaccharide part of sialyloligosaccharides on the host cell surface.

We further determined whether HA-pep25 must be a specific length to exert its activity or whether specific residues contained within HA-pep25 are critical for its activity. To accomplish this, we created five C-terminally shortened peptides (Figure 3a), and none of them exhibited activity (Figure 3b), which suggests that the length of the peptide should be kept constant. We also utilized alanine scanning to identify critical residues within HA-pep25 (Figure 3a). In the presence of eight mutant peptides, the HA(VN/04)-mediated influenza entry level was >50%, whereas HA-pep25 blocked >80% of the entry (Figure 3c). Thus, these eight residues (Ser_1, Lys_2, Arg_9, Phe_{12}, Trp_{14}, Thr_{15}, Ile_{16}, and Lys_{18}) are essential for HA-pep25's activity. Additionally, four peptides, which possessed individual alanine mutations at residues Asn_4, Gln_6, Met_{10}, and Phe_{13}, showed equivalent activity to HA-pep25. Therefore, these residues may potentially be used to optimize the physical and chemical properties of the peptide.

The HA-pep25 sequence is located at residues 221−238 in HA1 and includes the conserved 220 loop (residues 221−228) on the HA RBD.[8] The secondary structure of this segment in the crystal structure of HA (PDB 2FK0) is a β-strand. Therefore, we evaluated the secondary structures of HA-pep25 and the alanine-scanned peptides using circular dichroism (CD) spectroscopy (Figure S4). HA-pep25 possessed secondary structural features, and its maximum negative peak was located at approximately 214 nm. The spectrum of peptide m11 (Phe_{13}→Ala), which maintained anti-influenza activity, had a shape similar to that of the spectrum of HA-pep25. The peptides m10 (Phe_{12}→Ala), m12 (Trp_{14}→Ala), and m13 (Thr_{15}→Ala), which had no anti-influenza activity, had no secondary structural properties. Although the secondary structural elements identified in HA-pep25's spectrum did not perfectly match those present in the crystal structure of HA, these results suggest that maintaining a certain degree of structure is required for the peptide's activity.

In summary, we identified an HA-derived peptide, HA-pep25, that exhibited anti-influenza activities against both human and avian influenza viruses (IC_{50} = 12.0−51.0 μM). As a viral entry inhibitor, HA-pep25's activity was not as strong as those of neuraminidase inhibitors, such as oseltamivir carboxylate (IC_{50} = 0.5 μM); however, it produced stronger activity to ribavirin (IC_{50} = 65.0 μM), a replication inhibitor. As an entry inhibitor, HA-pep25 can be considered a drug candidate that may be used with other antivirals to target multiple steps of the influenza viral life cycle. Mechanistically, HA-pep25 blocked influenza viral entry by shielding the host receptor on the cell membrane. Furthermore, HA-pep25 does not bind to sialic acids present on host cell membrane, which explains its cross-species activity. The region

DOI: 10.1021/acsinfecdis.5b00139
ACS Infect. Dis. 2016, 2, 187−193

that HA-pep25 corresponds to in HA belongs to the receptor binding domain. Secondary structural changes by alanine substitutions caused a loss in activity.

In this study, HA-pep25 was derived from viral HA without modification. In future studies, HA-pep25 may be amenable to sequential optimization via a circular peptide strategy or through chemical modification, which may further augment its inhibitory activity.

METHODS

Peptide Synthesis. All peptides were synthesized using standard 9-fluorenylmethoxycarbonyl (Fmoc) chemistry methods on an automated SYMPHONY 12-channel peptide synthesizer (Protein Technologies, Inc.). Peptides were purified using reversed-phase high-performance liquid chromatography (RP-HPLC) and then lyophilized. The purity (>90%) and molecular weight of each peptide were verified by RP-HPLC and electrospray ionization−mass spectrometry (Shanghai China-Peptides Co., Ltd.).

Cells and Plasmids. Human embryonic kidney 293T cells and MDCK cells were obtained from the American Type Culture Collection (ATCC) and were maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum and 1% penicillin−streptomycin (Gibco). The cells were cultured at 37 °C and 5% CO_2. The plasmid encoding the HA gene (A/Vietnam/1203/2004, H5N1) in the pcDNA3.1(+) vector was a kind gift from Dr. Lijun Rong (University of Illinois at Chicago, Chicago, IL, USA). The plasmid encoding the HA gene (A/Goose/Qinghai/59/2005, H5N1) was kindly provided by Dr. George F. Gao (Institute of Microbiology, Chinese Academy of Sciences). The plasmid encoding the NA gene (A/PuertoRico/8/1934, H1N1) in the pEF6/V5-His-TOPO vector was kindly provided by Dr. John C. Olsen (University of North Carolina, Chapel Hill, NC, USA). The HA and NA genes derived from the influenza virus (A/Duck/Anhui/SC702/2013, H7N9) were synthesized by TSINGKE Biotech (Beijing, China) and were inserted into the pcDNA3.1(+) vector. The HIV vector (pNL4-3.Luc.R^-E^-) containing a luciferase reporter gene was obtained from the National Institutes of Health AIDS Research and Reference Reagent Program (Germantown, MD, USA).

Virus Preparation. Pseudotyped influenza viruses were produced as previously described.[25] Briefly, the plasmids encoding HA and NA and the HIV vector (pNL4.3.Luc-R^-E^-) were cotransfected into 293T producer cells using jetPEI transfection reagent (Polyplus). Forty-eight hours post-transfection, the cell supernatants containing the HA/HIV pseudovirions were collected and filtered through a 0.45 μm pore size filter (Millipore). These supernatants were used to directly infect 293T cells. The model control VSVG/HIV pseudovirions with the envelope protein of vesicular stomatitis virus glycoprotein (VSV-G) were simultaneously prepared. The live influenza virus (A/PuertoRico/8/1934, H1N1) stocks were kindly provided by Dr. Jianwei Wang (Institute of Pathogen Biology, Chinese Academy of Medical Sciences). The 50% tissue culture infectious dose ($TCID_{50}$) was determined according to a previously described protocol.[30]

Infection Assay. To screen the HA fragment peptide library, the peptides were dissolved in phosphate-buffered saline (PBS) without Ca^{2+}/Mg^{2+} at a concentration of 1 mM and were stored overnight at 4 °C. One day before infection, 293T cells were inoculated into a 24-well plate with 5×10^4 cells per well. After 24 h, the cells were incubated with the peptides at a final concentration of 100 μM for 1 h at 37 °C and 5% CO_2. Then, the cells were infected with HA(VN/04)/HIV pseudovirions, which mimicked the entry of highly pathogenic H5N1 influenza virus. After 48 h of infection, the cells were lysed, and luciferase activity was measured using an FB15 luminometer (Berthold Detection Systems) and a luciferase assay kit (Promega). The peptides that showed effective inhibition of HA/HIV viral entry were tested with the VSVG/HIV pseudovirions, and the specific antiflu peptide inhibitors were identified. To further assess the activity of HA-pep25 using the H1N1(PR8) viruses, the MDCK cells were inoculated into 96-well plates at 3×10^4 cells/well. After 24 h, the MDCK cells were washed twice with PBS to remove the serum and were then infected with 100× $TCID_{50}$ H1N1(PR8) viruses at 37 °C and 5% CO_2 for 2 h. After another rinse with PBS, the infected cells were incubated with peptide solutions at different concentrations for 72 h, and the cytopathic effects (CPE) observed in the cells were recorded. The 50% inhibitory concentration (IC_{50}) was calculated using the Reed−Muench method.[30] Each experiment was repeated three times.

Cell Binding Assay. The biotinylated HA-pep25 or biotinylated HA-pep3 were incubated with MDCK cells for 1 h at 37 °C and 5% CO_2. After five washes with PBS, the cells were incubated with horseradish peroxidase (HRP)-conjugated streptavidin (37 °C and 5% CO_2) for 15 min at a dilution of 1:200. The peroxidase activity was detected using 3,3′,5,5′-tetramethylbenzidine, and the reaction was stopped by adding 0.2 M H_2SO_4. Absorbance was measured at an optical density of 450 nm with a reference density of 620 nm.

Neuraminidase Digestion Assay. To assess whether the peptides could bind to sialic acid-free cells, MDCK cells were treated with a neuraminidase (New England Biolabs) derived from *Clostridium perfringens*, which can hydrolyze the α2,3-, α2,6-, and α2,8-linked sialic acids on glycoconjugates.[27,31] Briefly, MDCK cells were incubated with 500 U/mL neuraminidase for 1.5 h in G1 buffer (5 mM $CaCl_2$, 50 mM sodium acetate, pH 5.5; this was the reaction reagent suppplied with NA and was used as a reagent control) at 37 °C and 5% CO_2 followed by three washes with PBS. The cell surface α2,3- and α2,6-linked sialic acids were tested by *Sambucus nigra* lectin (SNA; specific for α2−6 linkages) and Maackia amurensis lectin II (MAL II; specific for α2−3 linkages) (Figure S5). The binding assay was performed by using the same procedure as described above for untreated MDCK cells, and the experiment was repeated three times.

Immunohistochemical Assay. For the immunohistochemical staining, mouse lung tissues were collected and fixed in 10% neutral buffered formalin. Then, the tissues were dehydrated, embedded in paraffin, and cut into 4 μm thick sections. After the sections were deparaffinized with xylene and rehydrated through a series of graded alcohol solutions, the sections were treated with 3% H_2O_2 at room temperature for 10 min to block endogenous peroxidase activity. Antigen retrieval was achieved by microwaving the sections in 10 mM citrate buffer (pH 6.0). The biotinylated peptides were then incubated with the sections at 4 °C overnight, after which the slides were rinsed with PBS three times. The slides were then incubated with the HRP−streptavidin solution at a dilution of 1:200 for 15 min at room temperature. The 3,3′-diaminobenzidine (DAB) substrate solution was applied to the sections on the slides for 2 min to reveal the color of the biotinylated peptides. After the slides were counterstained by immersing them in hematoxylin for 1 min, the slides were rinsed with running water and dehydrated through a series of graded alcohol solutions and xylene. The mounted slides were observed under a microscope (Olympus IX71).

DOI: 10.1021/acsinfecdis.5b00139
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SPR Analysis. The binding of HA-pep25 to sialyllactose was evaluated on streptavidin chips (SA chips) using SPR and a Biacore T200 Biosensor Processing Unit (GE Biosystems). The SA chip was preconditioned with three consecutive 1 min injections of 1 M NaCl in 50 mM NaOH. Then, the multivalent, biotinylated 3′-sialyllactose−PAA (Glycotech) was diluted to 10 μg/mL in HBS-P (10 mM HEPES, 150 mM NaCl, 0.005% surfactant P20, pH 7.4) and was immobilized on flow channel 2 (Fc2). The biotinylated PAA was immobilized as the reference control on flow channel 1 (Fc1). The immobilization levels of the ligand for each channel were approximately 350 response units. The running buffer used for the interaction analysis was PBS (pH 7.4). HA-pep25 was diluted in PBS to create the following concentrations: 3.75, 7.5, 15, 30, and 60 μM; HA-pep3 was also tested at 100 μM. The peptides were injected over the flow channel surface at 30 μL/min. The binding time of the peptides was 200 s, and the dissociation time was 200 s. The surfaces were regenerated with a 20 s injection of 50 mM NaOH at 30 μL/min and then washed with running buffer for 200 s.

Circular Dichroism Spectroscopy. Far-UV circular dichroic spectra studies were performed using a Jasco-815 spectropolarimeter. All spectra were recorded at 20 °C with constant N_2 flushing and a bandwidth of 2.0 nm in a quartz cuvette with a 1 mm path length. Wavelength scans were obtained in duplicate using a 0.2 nm step size and were averaged from three scans with a scan rate of 50 nm/min from 200 to 260 nm. The peptides were dissolved to a stock concentration of 1 mM in PBS (pH 7.4) and were diluted to 200 μM when tested.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsinfecdis.5b00139.

Information on our peptide library construction, HA sequence alignment, binding assays, CD spectra data, lectin detection assay, and SPR assay (PDF)

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Author Contributions

Y.G. directed the study. Q.C. performed the experiments. Y.G. and Q.C. wrote the manuscript.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

This work was supported by the National Natural Science Foundations of China (81202568, 81473256, and 81273561), the National Science and Technology Major Project (2015ZX09102-023-004), and the Specialized Research Fund for the Doctoral Program of Higher Education (20111106120022).

■ ABBREVIATIONS

HA, hemagglutinin; NA, neuraminidase; IC_{50}, 50% inhibitory concentration; PBS, phosphate-buffered saline; DMEM, Dulbecco's modified Eagle's medium; HIV, human immunodeficiency virus; VSVG, vesicular stomatitis virus glycoprotein; VN/04, highly pathogenic H5N1 virus (A/Vietnam/1203/2004); PR8, influenza virus (A/PuertoRico/8/1934, H1N1); MDCK, Madin−Darby canine kidney cells; CPE, cytopathic effects; $TCID_{50}$, 50% tissue culture infectious dose; RBD, receptor binding domain; SPR, surface plasmon resonance; RP-HPLC, reversed-phase high-performance liquid chromatography; HRP, horseradish peroxidase; DAB, 3,3′-diaminobenzidine; SA chips, streptavidin chips; CD, circular dichroism

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