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Inhibitory activity and mechanism of two scorpion venom peptides against herpes simplex virus type 1

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ABSTRACT

Herpes simplex virus type 1 (HSV-1) is a widespread human pathogen that causes severe diseases, but there are not effective and safe drugs in clinical therapy besides acyclovir (ACV) and related nucleoside analogs. In this study, two new venom peptides from the scorpion *Heterometrus petersii* were identified with effective inhibitory effect on HSV-1 infection *in vitro*. Both Hp1036 and Hp1239 peptides exhibited potent virucidal activities against HSV-1 (EC_{50} = 0.43 ± 0.09 and 0.41 ± 0.06 μM, respectively) and effective inhibitory effects when added at the viral attachment (EC_{50} = 2.87 ± 0.16 and 5.73 ± 0.61 μM, respectively), entry (EC_{50} = 4.29 ± 0.35 and 4.32 ± 0.47 μM, respectively) and postentry (EC_{50} = 7.86 ± 0.80 and 8.41 ± 0.73 μM, respectively) steps. Both Hp1036 and Hp1239 peptides adopted α-helix structure in approximate membrane environment and resulted in the destruction of the viral morphology. Moreover, Hp1036 and Hp1239 peptides entered Vero cells and reduced the intracellular viral infectivity. Taken together, Hp1036 and Hp1239 peptides are two anti-viral peptides with effective inhibitory effect on multiple steps of HSV-1 life cycle and therefore are good candidate for development as virucides.

1. Introduction

Herpes simplex virus type 1 (HSV-1) is a widespread human pathogen that infects primarily epithelial tissues and causes severe diseases including mucocutaneous lesions in the oral mucosa (cold sores), encephalitis, meningitis, and blinding keratitis (Gopinath et al., 2012). After an initial infection, HSV-1 spreads to the nervous system and establishes latent infection of neurons in sensory ganglia of the host (Hill et al., 1996). Approximately 80% of the world populations are carriers of HSV-1 and about 40% suffer from recurrent infection (Gold and Corey, 1987; Palem et al., 2011). Current therapeutic drugs against HSV infection are nucleotides, nucleosides or pyrophosphate analogues, such as acyclovir, valacyclovir, penciclovir and famciclovir (Hsiang and Ho, 2008). After uptake by virus-infected cells, these drugs are activated by the viral thymidine kinase and inhibit the viral DNA polymerase. However, HSV infection remains a serious challenge because of the viral resistance and side effect (Crute et al., 2002; Field, 2001). Therefore, the development of new safe and effective anti-HSV molecules is urgently needed.

Many antimicrobial peptides (AMPs) have been shown to have inhibitory activities against HSV infection. According to the structure characteristics, these peptides are classified into five types: α-helix, β-sheet, cyclic β-sheet, β-turn and extended (Jenssen et al., 2006). Proposed anti-viral mechanism of α-helix mainly includes cellular target and viral inactivation, including Magainin, Cecropin, Mellitin, LL-37 and Brevinin-1(Aboudy et al., 1994; Albiol Matanic and Castilla, 2004; Yasin et al., 2000). Two kinds of β-sheet peptides, human and rabbit defensins, were shown to interact with HSV membrane/glycoprotein and cellular targets but not heparan sulfate (Sinha et al., 2003; Yasin et al., 2004). Another β-sheet peptide from frog, dermaseptin, was shown to have potent inhibitory effect when applied to the virus before or during virus adsorption to the target cells (Belaid et al., 2002). Two β-sheet peptides tachyplesin and protegrin were proved to have viral inactivating effect (Yasin et al., 2000). Cyclic β-sheet peptides such as θ-defensin was shown to bound to gB protein and blocked HSV-1 attachment (Yasin et al., 2004). The β-turn peptides lactoferrin (LF) and lactoferricin (Lfcin) are antimicrobial peptides that were found to block HSV entry into Vero cells. LF had no effect against HSV after the virus had entered the cells, while Lfcin exerted anti-viral activity also after the initial binding of the virus to the host cells (Andersen et al., 2004; Jenssen et al., 2004). An extended peptide from bovine, indolicidin, showed a direct inactivation effect on cell-free HSV-1 virons by targeting viral membrane/glycoprotein (Albiol Matanic and Castilla, 2004).

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Natural AMPs from scorpion venoms have attracted much attention due to their anti-viral bioactivities. Some of them have been identified as anti-enveloped virus agents in our previous works. The peptide mucroporin-M1 was shown to be virucidal against the measles, SARS-CoV and influenza H5N1 viruses, and it inhibited HBV replication *in vitro* and *in vivo* (Li et al., 2011; Zhao et al., 2012). A natural a-helical peptide, Hp1090, was proven to have the property of killing HCV (Yan et al., 2011). Two histidine rich peptides were designed on the molecular template of a short virucidal peptide Ctry2459 and were confirmed to have enhanced bioavailability, which resulted in potent inhibitory effect on HCV proliferation (Hong et al., 2013). Another mutational peptide, Kn2-7, was also effective in inhibiting HIV-1 infection (Chen et al., 2012). These studies indicated that scorpion venom is a rich source of anti-viral peptides. In the present study, we screened and identified two peptides from the venom of scorpion *Heterometrus petersii*, which exhibited effective inhibitory effect on HSV-1 infection.

2. Materials and methods

2.1. Chemical synthesis

The Hp1035, Hp1036, Hp1165, Hp1239, Hp1412, Hp1478 peptides were from the non-amplified cDNA library of *H. petersii* venom gland that was constructed in our previous work (Ma et al., 2010). Peptides were chemically synthesized using the solid-phase synthesis method and amidated at the C-terminus (GL Biochem Ltd., China). The synthetic peptides were confirmed by RP-HPLC and MALDI-TOF-MS (purity > 95%).

2.2. Cell and virus

African green monkey kidney cells (Vero) were grown in minimum essential medium (MEM) supplemented with 10% (vol/vol) fetal bovine serum (FBS), 100 U/ml penicillin, and 100 μg/ml streptomycin sulfate at 37 °C in a 5% CO_2 incubator. Cells infected with virus were grown in MEM supplemented with 2% serum. To prepare high-titer stocks of HSV-1 (F strain) virus, Vero cells were infected at a low multiplicity of infection (MOI) of 0.1 in a T25 flask (NEST Biotechnology Co. Ltd. China). When the cytopathic effect (CPE) was 90–100%, the infected monolayers were harvested and subjected to three freeze–thaw cycles using a dry ice–ethanol bath, centrifuged at 1,000*g* for 5 min to remove debris, and stored at −80 °C. Viral titers were determined by plaque forming assay on Vero cells.

2.3. MTT assay

Cells were seeded in a 96-well plate (7000–10,000 cells per well) and cultured at 37 °C for 24 h. A series of concentrations of peptides were added into the medium, and the plate was incubated for at 37 °C for 48 h, at which time 20 μl of MTT solution (5 mg/ml in PBS buffer; Invitrogen) was added to each well, and the plate was incubated at 37 °C for 4 h. The medium was removed, 100 μl DMSO was added, and then the plate was shaken for 20 min at room temperature to completely dissolve the crystal purple formazan. The absorbance was measured at 570 nm.

2.4. Hemolysis

Freshly obtained human red blood cells were washed three times with HEPES buffer (pH 7.2) by centrifugation for 10 min at 1200*g*. The cells were then resuspended in 0.9% saline and seeded in a 96-well plate with 10^7–10^8 cells per well. A series of concentrations of peptides were added and incubated at 37 °C for 1 h. A 0.9% saline solution was used as a negative control, and 0.1% Triton X-100 was used as a positive control. The plate was centrifuged for 5 min at 1000*g*, and the absorbance of hemoglobin released in the supernatant was measured at 570 nm.

2.5. Plaque forming assay

Six-well plates containing Vero cell monolayers with about 85% confluence were infected with virus. After 1 h of absorption at 37 °C, the inocula were removed. Cells were washed three times with phosphate-buffered saline (PBS) and replenished with a maintenance cover layer (MEM with 2% FBS and 0.75% carboxymethylcellulose). After an incubation period of 3 days, the cells were stained with 1% crystal violet containing 10% methanal and the plaques were counted. The viral titer was calculated according to the plaque number and dilution.

2.6. Anti-viral assay

Six-well plates containing Vero cell monolayers with about 85% confluence were infected with virus to yield about 60 plaques per well. The anti-viral effects of peptides were determined in the following assays.

2.6.1. Viral inactivation assay

Peptides at appointed concentrations were incubated with virus at indicated conditions. At the indicated time, the virus–peptide mixtures were diluted and added to Vero cell monolayers. After 1 h of absorption at 37 °C, the inocula were removed. Cells were rinsed and replenished with cover layer as described above. After 3 days, the inhibitory effects were determined by plaque reduction assay.

2.6.2. Cell inactivation assay

Vero cells were incubated with peptides for 1 h at 37 °C, at which time the peptides were removed and the cells were washed with PBS for three times. The peptide-treated cells were then infected with virus at 37 °C for 1 h. Cells were rinsed and replenished with cover layer as described above. After 3 days, the inhibitory effects were determined by plaque reduction assay.

2.6.3. Viral attachment assay

Vero cells were cooled at 4 °C for 30 min and peptides were added to Vero cells together with virus at 4 °C for 1 h. At the indicated time the cells were rinsed with PBS for three times and shifted to 37 °C for viral entry. After 1 h incubation, cells were rinsed and replenished with cover layer as described above. After 3 days, the inhibitory effects were determined by plaque reduction assay.

2.6.4. Viral entry assay

Vero cells were infected with virus at 4 °C for 1 h, at which time cells were rinsed with PBS for three times and replenished with complete MEM containing peptides. Cells were then shifted to 37 °C for viral entry. After 1 h incubation, cells were rinsed and replenished with cover layer as described above. After 3 days, the inhibitory effects were determined by plaque reduction assay.

2.6.5. Postentry assay

Vero cells were infected with virus at 37 °C for 1 h, at which time the cells were rinsed with PBS for three times and replenished with cover layer containing peptides. After 3 days, the inhibitory effects were determined by plaque reduction assay.