Host cell targeting with peptide candidates derived from a HSV-1 entry protein

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Introduction

Herpes Simplex viruses (HSV-1 and HSV-2 of *Alphaherpesvirinae*) have unique entry mechanism into the host cells using their gD glycoprotein as well as gB, gC and gH/gL, as usual in case of other herpes viruses. gD glycoprotein selectively binds nectin-1 adhesion protein on the surface of the host's neurons, keratinocytes or epithelial cells followed by major conformational changes of the gD glycoprotein [1]. Based on the literature [2] and knowing the 3-dimensional structure of the HSV-1 gD and of HSV-1 gD – nectin-1 [3] receptor complex, we have chosen 20mer HSV-1 gD fragments represented by synthetic peptides for cellular uptake studies.

Methods

Overlapping 20-mer peptide amides of the HSV-1 gD 239-280 region were prepared on either SynPhaseTM lanterns (Mimotopes) or Rink-amide-MBHA resin with Fmoc/tBu strategy with DIC/HOBt chemistry. Methionine residues were substituted by norleucine to avoid oxidation. The peptides were acetylated for ECD measurements or labelled with 5(6)-carboxyfluorescein (Cf) for cellular uptake studies, and then cleaved from the solid support with TFA and appropriate scavengers, purified by RP-HPLC and characterised by ESI-MS. Carboxyfluorescein labelled peptides were characterised by fluorescence spectroscopy as well on different pH values.

Cellular uptake of Cf-HSV peptides into host cell model [4-6], SH-SY5Y human neuroblastoma cells [7-9] was characterised by the enhanced intracellular fluorescence measured by BD LSR II flow cytometer (Coherent Sapphire laser; $\lambda = 488$ nm, 22 mW) (incubation time: 3h; channel FITC LP505; BP 530/30), data were analysed with FACSDiva 5.0 software. All measurements were performed in duplicates, and the Cf-positive (Cf+) live cells with standard error of the mean was presented (Figure 1). Uptake of HSV 253-272 at 10 μ M was visualised in an Olympus CKX41 microscope.

Electronic circular dichroism (ECD) spectroscopy measurements were performed in the far-UV region on selected peptides with Jasco J-715 / Jasco J-810* spectropolarimeters, at 0.1 cm / 0.02* cm path length in quartz cuvette; in 2,2,2-trifluoroethanol (TFE); TFE-water 1:1 (v/v); water solvents, in ~0.14 mg/mL / 0.5-0.7 mg/mL* peptide concentration.

Results

Significant differences have been observed between the cellular uptake of individual peptides. Peptides containing the 253-268 region of the HSV-1 gD glycoprotein (HSV-249-268 and 253-272) were observable in more than 50 % of the cells in 10 μ M concentration. Parallel with flow cytometry measurements, to visualise cell morphology after Cf-peptide treatment, microscopic images of trypsinised, washed and resuspended cells were also captured. Fluorescent signal was concentrated on the cytoplasm (data not shown).

Figure 1: Cellular uptake profile of Cf-labeled HSV-1 gD peptides into SH-SY5Y neuroblastoma cells

Figure 2: A) ECD spectra of acetylated HSV-1 gD peptides in TFE, Jasco J-810 spectropolarimeter. B) Localization of the studied region on the HSV-1 gD in ribbon structure (PDB id: 3SKU), the helical region 253-268 is represented in black ribbon.

The ECD spectral patterns clearly indicate that most of the peptides are stabilised in helical conformation which is the consequence of the helix promoting effect of TFE [10]. The spectroscopic data are in good agreement with the cell internalisation profile. The most efficient cellular uptake was observed for HSV-249-268 and 253-272 (Figure 2A), which exhibit pronounced helical folding in the membrane mimicking solvent TFE. Conversely, sequences associated with little helical character even in pure TFE such as HSV-239-258, 257-276 and 261-280 (Figure 2A) showed decreasing internalisation rates.

It can be concluded that the disorder-to-helix conformational transition of HSV peptides is a decisive structural feature for their cell entry mediated by biomembrane interactions. Taken together, our studies demonstrated that nectin-1 binding sequences of the HSV-1 gD glycoprotein can internalise into human SH-SY5Y neuroblastoma cells with high efficiency. The most potent peptides are corresponding to sequences on the FG-loop of HSV-1 gD, within that, a helical structure (Figure 2B). Therefore, these peptides might be promising candidates for the development of conjugates for antiviral drug delivery into HSV infected cells.

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