Biochemical and Biophysical Research Communications 453 (2014) 64–68

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

X-pep, a novel cell-penetrating peptide motif derived from the hepatitis B virus

Kristopher Montrose, Yi Yang, Geoffrey W. Krissansen *

Department of Molecular Medicine & Pathology, Faculty of Medical and Health Sciences, University of Auckland, Auckland, New Zealand

ARTICLE INFO

Article history:
Received 4 September 2014
Available online 22 September 2014

Keywords:
Cell-penetrating peptide
Stereoisomer
Cell-permeability
X-protein
Hepatitis B virus

ABSTRACT

Cell-penetrating peptides (CPPs) are able to penetrate the plasma membrane and gain access to the interior of any replicating or non-replicating cell, and are being considered as drug delivery agents. Here we describe the serendipitous discovery of a novel CPP motif (MAARLCCQ), designated X-pep, located at the extreme N-terminus of the X-protein of the hepatitis B virus. X-pep, and a C-terminally truncated form of the peptide (MAARL), readily penetrated HepG2 cells. Further truncation by removal of the terminal leucine residue impaired the cell-penetrating activity of peptide, indicating that MAARL is the active core of the peptide. X-pep is located adjacent to another CPP, namely Xentry, and like Xentry is unable to penetrate unactivated resting lymphocytes suggesting selective cell uptake. A D-isomeric form of the MAARL peptide was not cell-permeable, indicating that the cell-penetrating function of the peptide involves stereoselective interaction with a chiral receptor. The discovery of X-pep, which bears no resemblance to known CPPs, allows studies to be undertaken to determine additional characteristics of this novel CPP.

1. Introduction

Over the past three decades more than 100 short peptides, commonly referred to as cell-penetrating peptides (CPPs), have been discovered to be capable of translocating the plasma membrane of cells [1–6]. CPPs are generally 10–30 amino acid (aa) residues in length, and are either arginine-rich, amphipathic and lysine-rich, or hydrophobic [7]. Many have been shown to have the ability to deliver cargo molecules into intracellular compartments, without causing significant damage to the plasma membrane, thus making them attractive non-viral vectors for delivering biologically active molecules into cells [1–6].

We recently described the serendipitous discovery of an entirely new class of CPP represented by the short peptide Xentry (LCLRPVG) derived from the N-terminal aa 16–20 of the X-protein [8]. The X-protein is a short 154 aa residue protein of 17 kDa encoded by the hepatitis B virus (HBV) [9], which is necessary for viral replication, regulates cell apoptosis, and contributes to the development of HBV-induced hepatocellular carcinoma [10–13]. Xentry differs from previously described CPPs in that it is very short, being only 7 aa residues in length with the active cell-penetrating core comprising just the 4 aa LCLR [14]. Further, it is not arginine-rich, amphipathic and lysine-rich, or uniformly hydrophobic [8]. The ability of Xentry to permeate adherent cells is dependent on the ubiquitously expressed heparan sulphate proteoglycan syndecan-4 [8]. Xentry is unique amongst CPPs as it is unable to passively enter syndecan-deficient, non-adherent cells, such as resting blood cells [8]. This feature offers a therapeutic advantage as Xentry is not sequestered and diluted by blood cells when injected intravenously. Xentry was able to deliver proteins, antibodies and siRNA in a biologically active form to the intracellular compartment of cells, and to tissues in mice [8].

At the time of discovery of Xentry, we became aware of a second CPP sequence almost immediately adjacent to Xentry, located at the extreme N-terminus of the X-protein. Here we describe a preliminary report on some of the features of this novel CPP, which has been designated X-pep.

2. Materials and methods

2.1. Cells and peptides

The human HepG2 (liver cancer) and mouse TK-1 (thymic lymphoma) cell lines were obtained from the American Type Culture Collection (ATCC). The HepG2 cell line was propagated in

Abbreviations: aa, amino acid; CPP, cell-penetrating peptide; HBV, hepatitis B virus.

* Corresponding author.
E-mail address: gw.krissansen@auckland.ac.nz (G.W. Krissansen).

http://dx.doi.org/10.1016/j.bbrc.2014.09.057
0006-291X/

full MEM medium at 37 °C and 5% CO2, while TK-1 cells were propagated in full RPMI 1640 medium at 37 °C and 5% CO2. All peptides were synthesized by Peptide 2.0 Inc., Chantilly, VA. L-isomeric forms of peptides are written in uppercase, and D-isomeric forms in lowercase, according to convention.

2.2. Assay to test the cell-penetrating ability of peptides

The HepG2 cell line was seeded into 8-well chamber slides at 1×10^5 cells per well in MEM medium (Gibco, Life Technologies New Zealand Ltd) containing 10% FCS and penicillin (100 U/ml), streptomycin (100 μg/ml), and L-glutamine (0.29 mg/ml). The cells were cultured overnight at 37 °C in a 5% CO_2 atmosphere, and washed thrice with serum-free MEM medium. FITC-labelled L-isomeric peptides were diluted in 500 μl of MEM medium without FCS, whereas FITC-labelled D-isomeric peptides were diluted in the same media containing FCS as D-isomeric peptides are resistant to serum proteases. Peptides were added to cells at a final concentration of 10 μM, or as indicated. The cells were incubated for 3 h at 37 °C in a 5% CO_2 atmosphere, washed with PBS, fixed with 4% formaldehyde for 30 min, and washed thrice with PBS. A drop of Prolong Gold anti-fade reagent with DAPI (Invitrogen, Life Technologies New Zealand Ltd) was added, and the cells mounted and examined with a Nikon E600 fluorescence microscope or a Leica TCS-SP2 confocal microscope.

3. Results

3.1. The extreme N-terminus of the X-protein contains a cell-penetrating peptide motif

During an investigation of the functions of short peptides spanning the entire length of the X-protein, it was revealed that the FITC-labelled N-terminal peptide spanning aa residues 1–20 (MAARLCCQLDPARDVLCLRP) was spontaneously taken up within minutes by HepG2 cells in a similar fashion to the overlapping Xentry (LCLRPVG)-containing FITC-labelled peptide spanning aa residues 16–35 (LCLRPVGAESRGRPVSGPFG) (Fig. 1A). Fluorescence and confocal imaging localised the peptide to both the cytoplasm and nucleus (Fig. 1A). It was expected that peptide aa 1–20 would be cell-penetrating as it contains the active motif of Xentry (LCLR) at its C-terminus, however there remained the possibility that a second cell-permeable motif was contained within the N-terminal region of this peptide. The peptide was C-terminally truncated to remove Xentry to test this notion, giving the peptide aa 1–15 (MAARLCCQLDPARDV). Conventional and confocal microscopy revealed that the truncated peptide aa-1–15 at 10 μM penetrated HepG2 cells (Fig. 1B). The truncated peptide was divided into two short peptides, containing aa 1–8 (MAARLCCQ) and aa 9–15 (LDPARDV). Peptide MAARLCCQ was readily taken up by HepG2 cells at a final concentration of 10 μM, whereas peptide LDPARDV was not cell-permeable (Fig. 2). Peptide MAARLCCQ was divided to

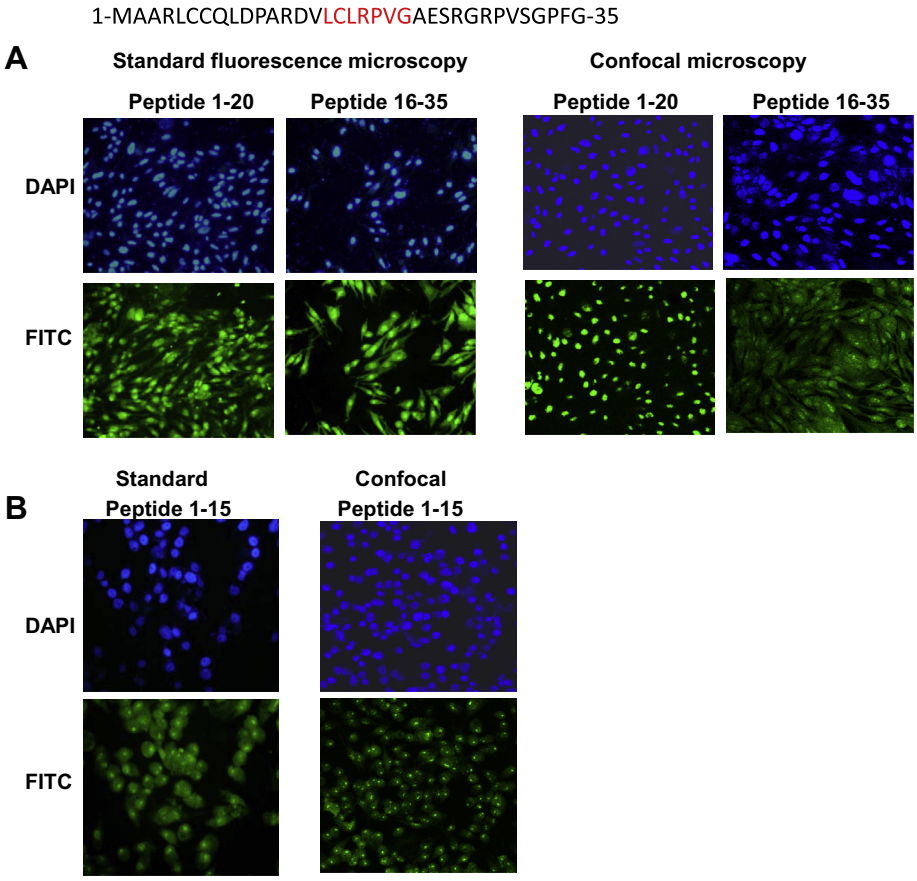

Fig. 1. The N-terminal X-protein peptide aa 1–15 is cell-permeable. The sequence of the first 35 N-terminal residues of the X-protein is shown with the sequence of Xentry (LCLRPVG; residues 16–22) highlighted in red. FITC-labelled peptides encompassing (A) aa 1–20 (MAARLCCQLDPARDVLCLRP) and 16–35 (LCLRPVGAESRGRPVSGPFG), and (B) aa 1–15 (MAARLCCQLDPARDV) from the N-terminal region of the X-protein were incubated with HepG2 cells for 3 h at a final concentration of 10 μM. Cell nuclei were stained blue with DAPI. Peptide uptake by the cells was recorded by standard fluorescence or confocal microscopy, as indicated. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)