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Impact of cell-penetrating peptides (CPPs) melittin and Hiv-1 Tat on the enterocyte brush border using a mucosal explant system

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ABSTRACT

"Cell penetrating peptides" (CPPs) are natural or synthetic peptides with the ability to interact with cell membranes in order to enter cells and/or deliver cargo. They attract considerable interest as permeation enhancers for oral delivery of therapeutic drugs with poor bioavailability, such as proteins or DNA. A main barrier is the intestinal epithelium where passage needs to proceed through a paracellular -and/or a transcellular pathway. Using an organ cultured mucosal explant model system and a selection of fluorescent polar -and lipophilic tracers, the aim of the present study was to investigate the interaction of two CPPs, melittin and Hiv-1 Tat, with the enterocyte brush border. Melittin belongs to the amphipathic class of CPPs, and within 0.5–1 h it bound to, and penetrated, the enterocyte brush border, causing leakage into the cytosol and increased paracellular passage into the lamina propria. Surprisingly, melittin also abolished endocytosis of tracers from the brush border into early endosomes in the terminal web region (TWEEs), excluding any permeation enhancing effect via such an uptake mechanism. Electron microscopy revealed that melittin caused an elongation of the brush border microvilli and a reduction in their diameter. HIV-1 Tat is a cationic CPP that is internalized by cells due to a sequence, mainly of arginines, from residue 49 to 57, and a peptide containing this sequence permeabilized enterocytes to a polar tracer by a leakage into the cytosol. In conclusion, the CPPs studied acted by causing leakage of tracers into the enterocyte cytosol, not by inducing endocytosis.

1. Introduction

"Cell penetrating peptides" (CPPs) is the collective term used for a multitude (~1000) of natural and synthetic peptides that possess the ability to interact with cell membranes to enter cells and/or deliver cargo [1,2]. They are generally less than 30 amino acids long and can be classified as either amphipathic, polycationic or hydrophobic on the basis of their physicochemical properties [3,4]. Their potential therapeutic value as permeation enhancers for poorly permeable drugs such as proteins and DNA has long spurred a wide pharmaceutical interest into how CPPs interact with target cell membranes to improve permeability without compromising safety concerns [1,4–7]. In particular, focus has been directed at their possible use in oral administration of macromolecular drugs, where the intestinal permeability barrier poses a major obstacle for efficient drug delivery [2,8,9]. However, regardless whether or not CPPs are covalently attached to their cargo, the mechanism whereby they increase epithelial permeability usually is not well understood. Two possible routes, a paracellular passage through tight junctions between neighboring cells and/or a transcellular pathway, may be enabled by CPPs, and the latter can occur via direct cell membrane translocation or by a number of different endocytic mechanisms [2,10,11].

In the present work we aimed to study how two different types of CPPs, melittin and HIV-1 Tat, interact with the small intestinal epithelium to promote the luminal uptake of a number of polar -and lipophilic fluorescent probes. Melittin is a 26 amino acid long natural, toxic peptide present in the venom of honey bees with biological effects including antimicrobial, antiparasitic and antifungal actions [12,13]. It belongs to the amphipathic class of CPPs, and X-ray crystallography data predicts melittin to consist of two α-helical regions separated by a non-α-helical segment at residues 11–12 [14] (Fig. 1). In aqueous solution at high ionic strength melittin forms tetramers with each monomer shaped like a bent rod [12]. It preferentially interacts with negatively charged membrane lipids and may adopt various orientations in the membrane, including both surface –and transmembrane locations, depending on transmembrane potential, lipid composition and peptide concentration. Melittin has long been known for its hemolytic action [15] which may occur via micellization or channel formation, although the molecular mechanism is not yet fully understood [12].

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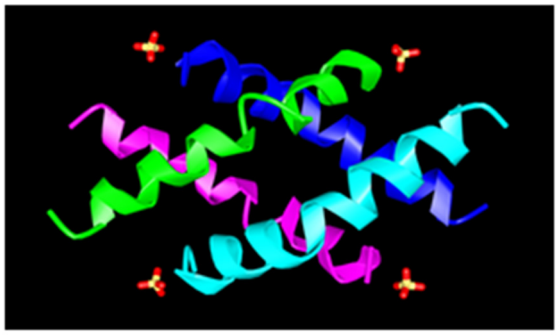
Melittin:

Gly-Ile-Gly-Ala-Val-Leu-Lys-Val-Leu-
Thr-Thr-Gly-Leu-Pro-Ala-Leu-Ile-Ser-
Trp-Ile-Lys-Arg-Lys-Arg-Gln-Gln



Melittin tetramer

Hiv-1 Tat peptide:

Tyr-Gly-Arg-Lys-Lys-Arg-Arg-Gln-Arg-Arg-Arg

Fig. 1. The amino acid sequence of melittin and the HIV-1 Tat peptide. The image shows the 3-dimensional structure of tetrameric melittin with four sulphate ions, based on crystallographic data [14]. (The image was downloaded from the PubMed Open Chemistry Data Base (https://www.ncbi.nlm.nih.gov/Structure/pdb/2MLT)).

Peptides derived from the 86-amino acid long transactivator of transcription protein of HIV-1 (HIV-1 Tat) belong to the class of cationic CPPs [16]. The ability to be internalized by cells relies on a short cationic sequence of mainly basic amino acids, mainly arginines, extending from residue 49 to 57 [16–18]. The polyarginine sequence is the essential motif, and the uptake mechanism has been proposed to involve a bidentate hydrogen-bonding between the guanidinium group of arginines and negatively charged groups of the membrane lipids [19,20]. Several studies on fluorescently labeled HIV-1 Tat peptides have indicated that this CPP is taken up into cells by an endocytic mechanism [16].

Our results, using an organ cultured mucosal explant model system, indicate that melittin bound to, and penetrated, the enterocyte brush border, causing a leakage of both polar and lipophilic tracers into the cytosol as well as an increased paracellular passage into the lamina propria. In addition, and more surprising, melittin abolished all endocytosis of tracers from the brush border into early endosomes in the terminal web region (TWEEs). A HIV-1 Tat peptide likewise permeabilized enterocytes to the polar tracer Lucifer yellow by leakage into the cytosol. Our work therefore indicates that these two CPPs act by causing leakage of tracers through the apical cell membrane rather than by endocytic uptake.

2. Materials and methods

2.1. Materials

Melittin from honey bee venom, a peptide (amino acids 47–57) derived from HIV-1 Tat (HIV-1 Tat peptide) and fluorescein 5(6)-isothiocyanate (FITC) were supplied by Sigma-Aldrich (www.sigmaaldrich.com), Lucifer yellow CH (ammonium salt) (LY), a

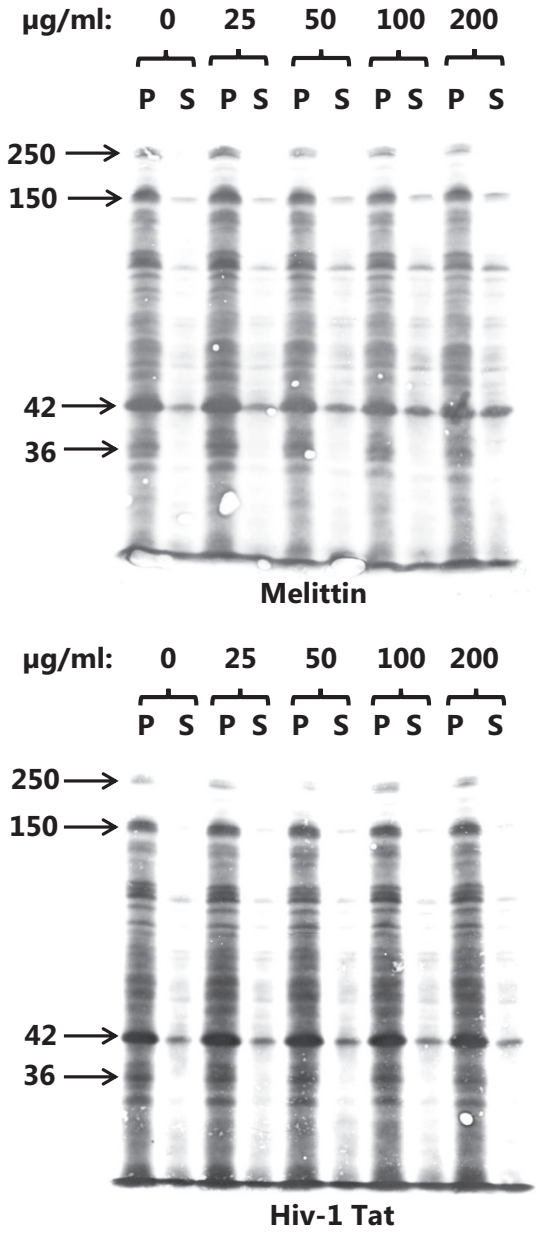


Fig. 2. Treatment of microvillus vesicles with melittin or the HIV-1 Tat peptide at a range of concentrations from 0 to 200 µg/ml. After treatment, the pellet (P) -and supernatant (S) fractions following a centrifugation were subjected to SDS/PAGE. Arrows indicate the molecular mass-values of the brush border proteins sucrase-isomaltase (250 kDa), ApN (150 kDa), actin (42 kDa), and annexin A2 (36 kDa).

fixable analog of the FM lipophilic styryl dye FM 1–43 FX (FM dye), Texas Red Dextran (MW 3000, lysine fixable) (TRD), ProLong antifade reagent with DAPI, and a monoclonal antibody to Na^+/K^+-ATPase (α-chain) by Thermo Scientific (www.thermodanmark.dk), and a mouse anti-rat early endosome antigen 1 (EEA-1) antibody by BD Transduction Laboratories (www.bdbiosciences.com), A rabbit antibody to pig intestinal aminopeptidase N (ApN) and the preparation of FITC-conjugated heat-labile enterotoxin from *Escherichia coli* (FITC-LTB) were described previously [21,22].

2.2. Animals

Animal experimentation in Denmark is subject to ethical evaluation by the Ministry of Justice's Council for Animal Experimentation. Animal