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Intracellular delivery of large molecules and small particles by cell-penetrating proteins and peptides

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Abstract

Cell-penetrating peptides (CPPs) have been used to overcome the lipophilic barrier of the cellular membranes and deliver large molecules and even small particles inside the cell for their biological actions. CPPs are being used to deliver inside cell a large variety of cargoes such as proteins, DNA, antibodies, contrast (imaging) agents, toxins, and nanoparticular drug carriers including liposomes. In this paper, we have reviewed the delivery of different molecules and particles mediated by TAT, Antp, VP22, and other CPPs as well as potential applications of these delivery systems in different areas of vaccine development, cancer immunotherapy, gene delivery, and cellular imaging.

Keywords: Intracellular delivery; Cell-penetrating peptides; Fusion proteins; Gene delivery; Toxins; Imaging agents; Particles; Liposomes

Contents

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1. Introduction

Many biologically active compounds, including various large molecules, need to be delivered intracellularly to exert their therapeutic action inside cytoplasm or onto nucleus or other specific organelles, such as mitochondria. However, the lipophilic nature of the biological membranes restricts the direct intracellular delivery of such compounds. Moreover, large molecules such as DNA, which are internalized via endocytosis [1] and transferred within endosomes, end in lysosomes resulting in the degradation of these molecules by lysosomal enzymes. So although many compounds show a promising potential in vitro, they cannot be used in vivo due to bioavailability problems. The methods like microinjection or electroporation used for the delivery of membrane-impermeable molecules are invasive in nature and could damage cellular membrane [2,3]. The non-invasive methods involve the use of pH-sensitive carriers including pH-sensitive liposomes [4], which under the low pH inside endosomes destabilize endosomal membrane liberating the entrapped drug into the cytoplasm.

A novel approach to deliver such molecules involves tethering them to peptides that can translocate through the cellular membranes, thereby enhancing their delivery inside the cell. During the last decade, several proteins and peptides have been found to traverse through the cellular membranes in a process called “Protein Transduction”, delivering their cargo molecules into the cytoplasm and/or nucleus. These proteins and peptides have been used for intracellular delivery of various cargoes with molecular weights several times greater than their own [5]. This process of protein transduction was discovered first by Green and Frankel independently, who found that 86-mer trans-activating transcriptional activator (TAT) from HIV-1 was efficiently taken up by various cells, when added to the surrounding media [6,7]. Subsequently, this property of translocation was found in Antennapedia (Antp), a transcription factor of *Drosophila* [8], and VP22, a herpes virus protein [9]. More precisely, their ability to translocate across the plasma membranes is confined to short sequences of less than 20 amino acids, which are highly rich in basic residues. Such sequences are called “Protein Transduction Domains (PTDs)” or “Cell-Penetrating Peptides (CPPs)”. Cellular delivery using CPPs has several advantages over conventional techniques because it is efficient for a range of cell types, can be applied to cells en masse, and has a potential therapeutic application [10].

The details on the nature of CPPs and their proposed mechanisms of translocation are discussed in other articles of this issue. However, we still will provide here a brief description of some of CPPs involved in delivery of therapeutic agents, and then focus mainly on applications of CPPs for various biomedical purposes.

2. Cell-penetrating peptides (CPPs)

CPPs are divided into two classes: the first class consists of amphipathic helical peptides, such as transportan and model amphipathic peptide (MAP), where lysine (Lys) is the main contributor to the positive charge, while the second class includes