Cell-Penetrating Peptides (CPPs): A tool in modern biotechnology

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ABSTRACT

The major limitation in the application of bioactive molecule is their low permeation across plasma membrane. In 1988 it was discovered, that a natural polycationic protein, the trans-acting activator of transcription (TAT) of the human immunodeficiency virus (HIV-1), passed very efficiently through cell membrane of cultured mammalian cells. TAT became known as the first cell penetrating peptide (CPP). CPPs have demonstrated themselves to be capable of delivering biologically active cargo to the cell interior and the vehicular capabilities of CPPs have already been harnessed for use as laboratory tools. Attached to a CPP, therapeutic cargo could be delivered to an intracellular target, thus overcoming the entry restrictions set by the plasma membrane. Since the discovery of TAT, the number of known peptides with cell-penetrating capabilities has grown and in 2003, the first CPP-based drug reached phase II clinical trials. In this review we introduce and discuss the current knowledge of CPPs.

Keywords: Cell penetrating peptides (CPPs); Plasma membrane; Drug delivery.

INTRODUCTION

Cell membrane as a selective permeability barrier surrounded the cells. While the phospholipid bilayer membrane play important role in cell function and survival, but often delivering specific material that we need into cells is a problem. Reporter molecules and imaging agents need to log into live cells for their performance, so find a way to bring them into cells is essential. In some cases, the chemical changes caused increased permeability of cells obtained, sometimes chemical changes to transfer material into cells cause lose or reduction efficiency of our material [1-3].

Adding synthetic vector is another method to increase the penetration into the cells. Among the various vectors that are present, cell penetrating peptides (CPPs) have become most popular and effective techniques for entry into cells. Usually CPPs are short cationic sequence peptide that may be derived from natural resources or designed synthetically [4, 5].

The first ability of CPPs was observed in 1988 by Frankel and pabo [6]. It was found that (TAT) protein of HIV-1 virus could enter into the cell and nucleus in cultured cells [6]. Further investigations shown for cell penetrating ability of TAT did not required whole sequence of TAT and small piece of amino acids sequence of the TAT is responsible to it and then named TAT peptide. The sequence of amino acids 49 to 57 (TAT_{49-57}) of the protein in question is causing this effect [7]. Using CPP strategy in 1994 when it was discovered that the third helix of *Antennapedia* transcription factor called pAntp (43-58) or penetratin alone has the ability to cross cell membrane [8]. Some of the CPPs maintain its ability to cross cell membranes even adding acidic or hydrophobic material to them. CPPs ability to deliver and pass molecules that cannot enter the cells single; they are also called Trojan peptides [9]. Researches on CPP peptides have continued to date and list of them increasingly on the rise (Table 1).

Table 1. Numbers of cell-penetrating peptides.

Peptide Name	Amino acid sequence	Source of peptide separation	Reference
Penetratin, pAntp	RQIKIWFQNRRMKWKK	*D. Melanogaster* transcription factor	[9]
HIV TAT peptide (49-57)	RKKRRQRRR	Viral transcriptional regulator	[7]
VP22 peptide	DAATATRGRSAASRPTERPRAPARSASR PRRVD	HSV-1 Viral Capsid protein	[10]
MAP (Model amphiphilic peptide)	KLALKLALKALKAALKLA-amide	Synthetic	[11]
Transportan	GWTLNSAGYLLGKINLKALAALAKKIL-amide	Chimeric galanin-mastoparan	[12]
R7	RRRRRRR	Synthetic	[13]
MPG	GALFLGWLGAAGSTMGAPKKKRKV	Chimeric HIV-1 gp41-SV40 large T antigen	[14]
Pep-1	KETWWETWWTEWSQPKKKRKV	Synthetic	[15]

Selection the appropriate CPP peptides, often depends on the purpose for which the researcher faces. Some commonly used peptides include TAT, polyargenin, penetratin and Transportan. CPP peptides, particularly in transport of proteins into cells so far have been successful [16]. Some application of CPPs with emphasis on the effects and uses of them in biotechnology are considered in this review.

Cellular Uptake of CPPs

Although, mechanism of CPPs passage through cytoplasmic membrane has been the subject for many studies, an integrated mechanism, which could explain this translocation, has still remained ambiguous. It has been suggested that some of characteristics of peptide are effective on its translocation and passage including molecular length and its static charge at the same time some of molecular properties which are accompanied to these peptides like size and electric charge may have several important impacts on mechanism for peptide uptake [17]. First of all, it was posited that these peptides could be displaced by direct translocation through plasma membrane. This model was presented based on biochemical and biophysical evidences [18]. Recently, it has been shown that endocytosis process may play an essential role in entry of cationic (basic) and amphipathic CPPs like *Antennapedia* (Antp), R9, and TAT proteins [19]. The study on various CPPs through several cellular classes showed that the mechanism of arrival depends on experimental conditions. In general, it can be implied that the mechanism of entry for these peptides can be classified into two groups: 1) Energy- dependent endocytosis; 2) Direct and energy- independent translocation through bi-laminar phospholipid membrane [20]. In study on CCPs endocytosis path, the investigation indicated that there is not only one endocytosis mechanism to enter these peptides through the cell, but macropinocytosis, clathrin-dependent endocytosis and Koala- dependent endocytosis might contribute to entry of these peptides under various conditions as well [21].

Passing of quantum-dots through Blood- Brain-Barrier (BBB)

Passing through Blood- Brain Barrier (BBB), which is made up a series of firm links among endothelial cells, is one of the problems for access to brain tissues. TAT peptide has been used to bring quantum-dots to cerebral tissue of the rat in order to penetrate into this barrier in one of these studies [22]. In this survey, a micro catheter was inserted in rat's cervical carotid aorta in order to reach conjugated TAT peptide to quantum-dot. TAT peptide could successfully and quickly reach quantum-dots into brain tissue where the rate of

its accumulation in cerebral tissue was to the extent that one could observe the florescence of rat's brain tissue by means of UV handheld torch with low power as well [22]. The interesting point in transferring quantum-dots through Brain-Blood- Barrier (BBB) without manipulation in animals is referred to this issue that these measures can be employed to identify human diseases tumors during surgical operation [22].

Application of CPPs to transfer cellular biosensors

In addition to transferring of scanning agents, CPPs may be also utilized in reaching light-emitting biosensors through the cells. Zn metallic element is cofactor for many enzymes, transcription factors, protein of immunity system and it is considered as the second rare elements in human body in terms frequency [23]. Although Zn is an important functional element, increase of this element is toxic under some conditions like disease, ischemic attacks, Alzheimer, and epilepsy. Thus, a method can be useful, which may measure and track rate of Zn metal and its distribution in body or quantity of the existing Zn in proteins. In the body, a great amount of Zn metallic ion is linked to some agents like proteins, glutathione, and histidine so very little amount of Zn is found freely and available for measurement [24]. For this purpose, fluorescent biosensors may be designed and developed to measure intracellular Zn surface (ruffling). Human carbonic anhydridase enzyme is used as biosensor to measure Zn and its link to TAT peptide may effectively cause its entry to cell without manipulation of cellular membrane [25]. Its signal appears quantitatively and it has been shown that it is also suitable for measuring Zn ionic surface up to less than 5-10 picomolars inside the nucleus and cellular cytoplasm. This new and sensitive biosensor may be employed to study on Zn ionic surface inside the cells as well as surveying Zn role in cellular biology [26, 27].

Transferring Peptide Nucleic Acids (PNAs)

Peptide Nucleic Acids (PNAs) are synthetic polymers, which are synthesized like DNA or RNA but they differ from those molecules in that they have peptide structure instead of glucose structure. During recent years, PNA oligomers have been adapted in process of molecular biology, diagnostic tests, and anti-sense therapeutic methods [28]. Transferring these compounds into the cells has been the major problem for their application because they lack charge. To improve entry inside the cells, some agents like receptor of certain ligand and or Nuclear Localization Sequence (NLS) were added to them [29]. In an investigation that has been conducted by Samir Al-Andalusia et al, they observed that application of Cell-Penetrating Peptide (M918) is effectively responsive to reach PNA inside the cell and it resulted in better outcomes compared to other techniques for entry into the cell [30].

CPPs for delivery of siRNA inside the cell

CPPs are used to transfer Small Interfering RNA (siRNA) inside the cells to regulate gene expression as well. The existing reports indicate that application of TAT peptides for transferring siRNA inside the cell was very effective and caused suppression of gene [31].

The recent studies have introduced a new CPP under title of Peptide for Ocular Delivery (POD) that can reach small and big molecules into the cells in ocular tissue [32]. POD peptide successfully enters siRNAs into human retinal stem (embryonic) cells and gene suppression has been seen more than 50% of cases. Local use of conjugated POD with color in retina of rats has also caused their ocular tissue to absorb conjugated paint [33]. In the future studies, this subject will be explored that if POD peptides can transfer medications into ocular cells (without side-effects) or not.

Local transfer of medications by CPPs

One of the applied aspects of connected molecules to CPP peptides, which can be used in clinical condition as well, is to used them directly on target cells and or at least adjacent to them. For instance, in one of the conducted studies, one can refer to entering cyclosporine A that is linked to a homopolymer from arginine, inside the cell [13]. In this study, passing through *stratum corneum*

and spreading inside epidermis have been observed. In 2003, this compound has entered to the second phase of clinical test of psoriasis disease under trade name of PsorBan®.

The other example is to intra-coronary injection of a compound from C-kinase protein inhibitor (δ) together with TAT peptide that has been well-known under title of KAI-9803 (a delta protein kinase C inhibitor) and it will be administered to treat several myocardial infarctions. Intra-coronary infusion of KAI-9803 has entered into phase-I of clinical studies on March 2007 [34]. Several applications for these peptides can be seen in Fig. 1.

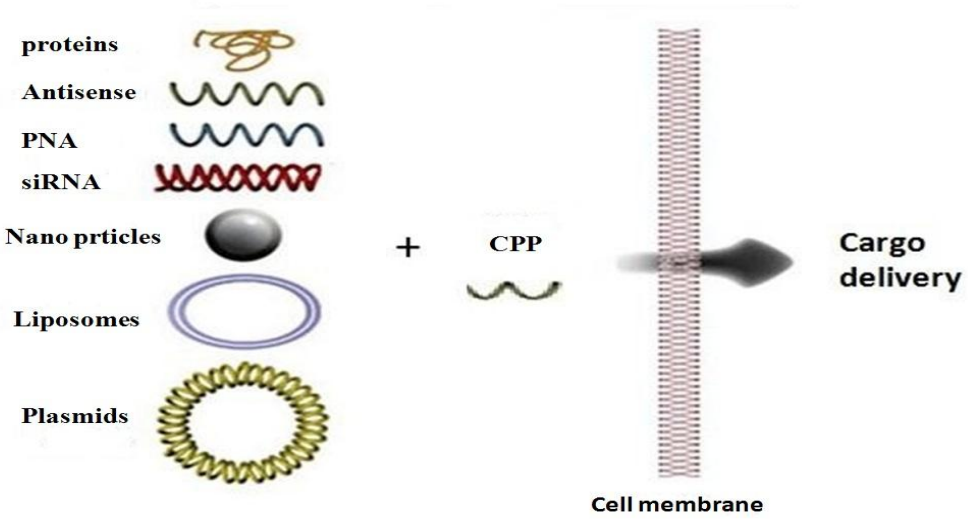

Figure 1. Different applications of membrane-penetrating peptides.

CONCLUSION

Cell-Penetrating Peptides (CPPs) have shown that they could be useful in transferring a wide range of bio-molecules into several cellular classes *in vitro* as well as *in situ* for living organisms. Many studies are in-process to develop their application as well as specialization of these peptides for the tissues and as it mentioned before, they could be efficiently useful in medical biotechnology.

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