

DEVELOPMENT OF AN EFFICIENT CYSTEINE RICH CELL PENETRATING PEPTIDE BY STRUCTURE ACTIVITY STUDIES

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Introduction: Cell Penetrating Peptides (CPPs) are potential tools for the intracellular delivery of wide range of cargos. Though the exact translocation mechanism is still unknown, endocytosis is the most prevalent uptake mechanism seen for highly cationic peptides. Release from endosomes for colocalization of cargo/drug and target in the cytoplasm is the major hurdle of targeting approaches. Therefore, there is a need for vectors capable for transferring cargo molecules directly into the cytoplasm. Herein, we focus on the development of a novel CPP derived from Crotoamine (polypeptide in venom of rattlesnake) which shows an efficient uptake at low concentrations ($\leq 2.5 \mu\text{M}$) and cytosolic distribution along with vesicular uptake.

Methods: Series of peptides were synthesized by Fmoc strategy, introducing mutations in Cro₍₂₇₋₃₉₎ (proposed CPP sequence in Crotoamine). All were labeled with fluorescein isothiocyanate at the N-terminal. SAR studies were done by substitution and/or deletion of amino acid residues in the sequence observing the uptake behaviour by fluorescence spectroscopy and microscopy.

Results: Amongst 61 synthesized peptides one of shorter length was showed the best intracellular delivery and cytosolic distribution. Replacing or deleting cysteines had negative impact on internalization. Results also show the involvement of tryptophans in cellular uptake indicating along with cationic amino acids the importance of each residue in this optimized sequence along with cationic amino acids.

Conclusions: SAR studies identified a peptide showing, besides of endosomal uptake, also an efficient delivery into the cytoplasm. Thus, this peptide might prove useful for efficient transmembrane delivery of agents directed to cytosolic targets.