

NOVEL PEPTIDE DELIVERING DIRECTLY INTO THE CYTOSOL: PROSPECTIVE TOOL FOR INTRACELLULAR TARGETING

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Introduction: Crossing the plasma membrane is a prerequisite for intracellular targeted drug delivery. Cell penetrating peptides (CPPs) are known to transport cargo molecules attached to it into cells primarily by endocytosis. Nevertheless, confinement of biomolecules into endosomes limits their use for intracellular targeting. We developed a novel cysteine-rich peptide that has the ability to enter directly into the cytosolic compartment of the cell. The factors affecting cytosolic distribution of peptide are reported.

Methods: Uptake of fluorescently labeled peptide was assessed in NIH-3T3 mouse fibroblasts. Effect of varying the labeling concentration, time course, changing incubation temperature from 37°C to 4°C, etc. on intracellular distribution was observed by fluorescence spectroscopy and microscopy.

Results: Our novel peptide exhibited mainly cytosolic localization along with some vesicular uptake in cells at a concentration as low as 2.5 µM. Diffused appearance was visible earliest after 4h. A reduction in vesicular uptake was observed on incubation at 4°C, however cytosolic uptake was almost unaffected indicating for an additional non-endosomal pathway. Due to higher stability, d-form of most of the well studied cationic CPPs (like Tat, arginines, etc.) is reported to be taken up better than the l-form. Our peptide differs as the l-form shows the most efficient cytosolic uptake while the d-form and retro-inverso form exhibited especially reduced cytosolic diffusion.

Conclusions: Thus, a novel cysteine-rich peptide has been found that is directly taken up into cytosol avoiding endosomal entrapment. Therefore, it has the potential to be used as CPP for efficient cytosolic drug delivery for intracellular targeting.