Cell Penetrating Peptides and their conjugates for optical and MR imaging techniques

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The prerequisite of intracellular contrast agents (CA) is the ability to cross the plasma membrane by different mechanisms like endocytosis. It is well documented that cell penetrating peptides (CPP) are the potential carriers of cargos through the plasma membrane. Arginine rich peptides are well known for their transduction behavior. Our goal is to find optimal and biocompatible CPP sequences for the efficient internalization of CA into the cells for Magnetic Resonance (MR) Imaging. Achieving this goal will allow the detection of specific targets (eg. enzyme reporters) within the cells by MR Imaging. Efficient internalization as well as release of the CA into the cytosol after endocytotic uptake is a necessary requirement for most of the intracellular targeted CA. The latter seems to be a bottleneck of this approach. In this study we used and modified well known CPP to achieve an efficient vector for intracellular targeting.

Regular and modified CPP were synthesized in solid phase by general Fmoc synthesis strategy on Wang resin. Coupling of fluorescein isothiocyanate (FITC) and gadolinium (Gd) chelator DOTA (tris-t-Bu-ester) was done on resin as well [1, 2]. In case of stearoyl modified CA [3] an additional lysine was introduced between the peptide and Gd chelator to link the fatty acid residue. CA were cleaved from resin and loaded with Gd in water. Compounds were purified by RP-HPLC, freeze-dried and analyzed by ESI-MS.

Modifications include exchanging of the natural L conformer of amino acids to D form (peptide D form is more resistant to enzymatic degradation), introduction of a fatty acid group (the hydrophobic moiety contributes to absorbing the molecule on the cell membranes), or substituting glutamine with ornithine in Tat_{57-49} peptide. All are discussed to enhance internalization.

A series of intracellular CA were synthesized showing efficient cell internalization in NIH 3T3 mouse fibroblasts. Satisfactory multistep syntheses for all CA with an overall yield in the range of 4% to 35 % were established. Fluorescence and MR Imaging studies showed that internalization efficiency and contrast enhancement of CA containing modified CPP was higher, but also combined with an increased cytotoxicity. Further optimization and modifications are required to achieve CA with high cellular uptake and biocompatibility.

References:
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