Efficient Intracellular Delivery of an MR Imaging Probe by a Novel Cell Penetrating Peptide

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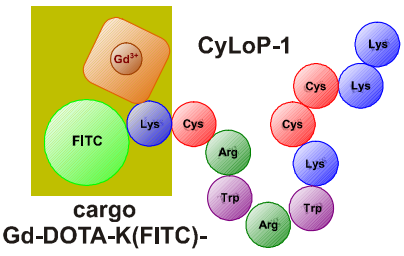
Introduction The quality of an intracellular targeted imaging probe or drug depends not only on the efficient delivery across the cell membrane but also on co-localization with its intracellular targets. In recent years, cell penetrating peptides (CPPs) have been investigated for their use as delivery tools. However, several studies reported on the entrapment of CPP conjugates into endosomes after uptake, thus, restricting their use for cytosolic targeting. Here, we are presenting the development of a novel cysteine rich CPP (derived from polypeptide crotamine (1)) able to distribute imaging probes into the entire cytosol.

Methods Series of peptides were synthesized by Fmoc strategy and were N-terminally labeled with fluorescein isothiocyanate (via an additional lysine (K) residue) for optical detection. After purification, conjugates were solved in water and concentration was determined using the absorbance of fluorescein. The peptide sequence was optimized by Structure Activity Relationship (SAR) studies with regard to cellular uptake as well as cytosolic distribution. Cellular uptake of compounds was confirmed by fluorescence microscopy and spectroscopy in NIH-3T3 mouse fibroblasts plated in 96well plates. Internalized fluorescence was measured in a multiplate reader, and microscopic images were made to evaluate cellular distribution of the peptides. Gd-DOTA was coupled to the peptide with the best uptake and highest cytosolic appearance via the α-amino group of the N-terminal lysine. Uptake and cellular distribution were tested again, and MR analyses of cells labelled with this conjugate were performed in Eppendorf tubes (1 x 10^7 cells/tube). MRI of cell pellets was conducted at 3T using T_1- and T_2-weighted spin-echo sequences. Relaxation rates were obtained from axial slices as well as T_1-weighted images of sagittal slices.

Results and Discussion 60 peptide derivatives were synthesized and characterized by ESI-MS. SAR studies revealed the best internalization properties for CyLoP-1 (Cytosol Localizing Peptide). It is a 10 amino acid long sequence containing cationic amino acids (common for various CPPs), cysteine, and tryptophan residues (Scheme). It is markedly distinct in its function, showing an efficient uptake at low concentrations (≤2.5 µM) and a cytosolic distribution along with vesicular uptake unlike other common CPPs (e.g. Tat or Antennapedia) at these concentrations (2).
Coupling of Gd-DOTA to CyLoP-1 (Gd-DOTA-K(FITC)-CyLoP-1) only slightly decreased the uptake whereas the cytosolic localization of the conjugate was maintained (Fig.1). Furthermore, uptake efficacy at 2.5 µM was significantly higher compared to a similar conjugate containing the *d*-form of Tat peptide, a well known efficient CPP.
In vitro labeling of 3T3 cells with the Gd containing conjugate resulted in a highly significant contrast enhancement in MR images already at low micromolar concentrations. The cellular relaxation rate $R_{1,cell}$ was increased to 245 and 425% of control at 2.5 and 5 µM, respectively (Fig. 2A). This effect was also clearly visible in the T_1-weighted images of cells (Fig. 2B). The contrast enhancement is the best we observed up to now for our intracellular contrast agents and is likely due to the distribution of this contrast agent complex in the entire cytosol, leading to a higher accumulation inside the cell, as well as access to a larger pool of water molecules, as compared to probes which remain entrapped in endosomal vesicles.
Thus, the novel peptide CyLoP-1 proved to be efficient in transmembrane delivery of imaging agents and is expected also to be useful as a vector for delivery of probes specifically targeted to cytosolic constituents.



Schematic structure of the synthesized conjugate of CyLoP-1and imaging

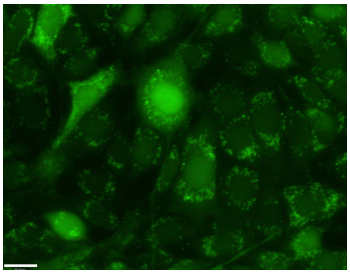


Fig. 1: Cellular distribution of Gd-DOTA-K(FITC)-CyLoP-1 in 3T3 cells.
Fluorescence microscopic image of 3T3 cells after labelling at 2.5 µM for 18h.

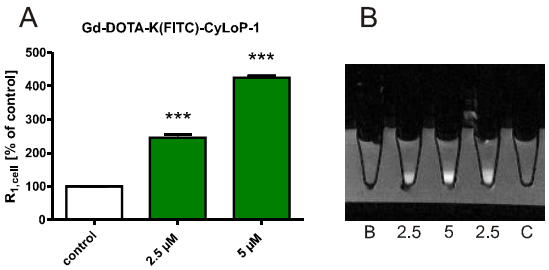


Fig. 2: Contrast enhancement of Gd-DOTA-K(FITC)-CyLoP-1 in 3T3 cells.
(A) Cellular relaxation rate $R_{1,cell}$ after labelling for 18h at indicated concentrations; (B) Corresponding T_1-weighted images. B, medium blank without cells, C, unlabelled control cells.

References (1) Kerkis A. *et al.*, *FASEB J.* 2004, *18*, 1407. (2) Duchardt F. *et al.*, *Traffic* 2007, *8*, 848.