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Cytosolic delivery of macromolecules using pH-dependent fusogenic peptide

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The plasma membrane plays critical roles in maintaining cellular homeostasis. It serves as a barrier against unfavorable attack on cells from an unpredictable external world. However, the membranes are also barriers to intracellular delivery of various therapeutic molecules. For improving their translocation, we developed a novel method using GALA peptide/cationic lipid complexes. The GALA peptide (amino acid sequence: WEAALAEALAEALAEHLAEALAEALEALAA) is a 30-residue amphipathic peptide with a repeat sequence of glutamic acid–alanine–leucine–alanine, and designed to mimic the function of viral fusion protein sequences that mediate escape of virus genes from acidic endosomes into cytosol [1]. The GALA peptide converts its structure from random to helical when the pH is reduced from 7.0 to 5.0, and this leads to destabilization of the membranes. When attached with bioactive cargoes, the GALA peptide may thus serve as intracellular vector bearing efficient endosomal escape function. However, the negative charges from glutamic acids (seven residues) in the GALA sequences reduce the efficiency of binding to a negatively charged cell surface. To overcome this problem, a cationic lipid was employed as an 'adhesive' for pasting the GALA peptide onto cell surface to accelerate its cellular uptake. We examined the ability of GALA peptide as a delivery vector using FITC as a model of membrane-impermeable low-molecular weight drugs. When FITC–GALA (1 μM) was administrated to HeLa cells, co-addition of cationic lipid, Lipofectamine 2000 (LF2000), significantly increased uptake efficiency. In a time-dependent manner, FITC–GALA escaped from endosomes, and diffuse fluorescent signals were observed in both cytosol and nucleus, suggesting that the cytosolic translocation proceeds along with endosomal acidification. The GALA/cationic lipid system was also applied for the intracellular delivery of FITC–avidin protein (68 kDa). When FITC–avidin (250 nM) was mixed with biotinylated-GALA (1 μM)/LF2000 complexes, FITC–avidin effectively internalized into cells, and diffuse signals of the FITC–avidin in cytosol were observed. In the absence of these complexes, efficiency of cytosolic diffusion of the FITC–avidin was quite low. These results suggest the usefulness of our approach for intracellular delivery of macromolecules using GALA peptide and cationic lipid [2].

Reference

1. Subbarao, et al. *Biochemistry* 1987;**26**:2964–72.
2. Kobayashi, et al. *Bioconjug Chem* 2009;**20**:953–9.

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A44
A dual uptake mechanism for the peptide Tat–LK15

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Knowing the mechanism of uptake is fundamental in developing new and refining existing drug delivery systems. Recently, Tat–LK15 peptide resulting from the fusion of Tat peptide and the synthetic amphipathic LK15 has shown greater transfection efficiency than Tat alone [1]. However, its uptake mechanism is ambiguous as the influence of the amphipathic peptide (LK15) and Tat peptide upon binding remains unclear. To elucidate this issue, the present study investigates the effect of temperature and peptide concentration on the cellular uptake mechanism of TAMRA–Tat–LK15 peptide. HeLa and HT29 cell lines were incubated with 1, 2.5 or 5 μM TAMRA–Tat–LK15 and TAMRA–Tat peptide solutions at different temperatures (4°C, 20°C, and 37°C) or in the presence of sodium azide (a metabolic inhibitor). A Zeiss LSM510 microscope was used to monitor cellular uptake in using a thermoelectric controlled temperature chamber. Our data indicate clearly TAMRA–Tat–LK15 peptide uptake (diffuse distribution in the cells) at 4°C for a 5 μM bulk solution while images do not suggest uptake in these conditions at lower concentrations (1 and 2.5 μM). At higher temperatures (20°C and 37°C) TAMRA–Tat–LK15 was observed in cells at all concentrations (a mixture of diffuse and punctuated fluorescence in cells). Interestingly, pre-incubation with 10 mM sodium azide did not completely block peptide uptake in cells for 1 and 2.5 μM bulk peptide solutions, and, had no effect at 5 μM and above. Overall, the results suggest the presence of concentration dependent uptake mechanisms of the Tat–LK15 peptide in cells.

Reference

1. Saleh AF, et al. Improved Tat-mediated plasmid DNA transfer by fusion to LK15 peptide. *J Control Release* 2010;**143**:233–42.

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A45
Visualizing the effect of integrin targeting and surface shielding on gene vector uptake by live cell imaging

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Nonviral vectors enable the safe delivery of transgenes into target tissues but are still less efficient than viral gene vectors. To develop novel artificial systems with enhanced efficiencies a detailed understanding of the cellular uptake and intracellular trafficking is essential. By visualizing the entire pathway of a single nanoparticle, from its first contact with the cell surface to the delivery of the DNA to the cell nucleus, detailed information about the mechanisms of uptake, intracellular trafficking and DNA release can be gained. Here we study the effect of integrin targeting and surface shielding on the internalization of gene vectors by live cell imaging with highly sensitive fluorescent microscopy. αvβ3 and αvβ5 integrin receptors are attractive targets for antiangiogenic cancer gene therapy as they play a pivotal role in angiogenesis and proliferation of malignant tumors. A cyclic RGD peptide specifically binds to those receptors and thus can be used for specific targeting of gene vectors such as polyplex micelles. In this study the analyzed micelles consisted of a thiolated PEG-block-poly(lysine) copolymer complexed with fluorescently labeled DNA [1]. To analyze the influence of shielding, two types of micelles containing a differently sized PEG shell were compared. To directly compare the internalization of targeted and untargeted micelles without knowing the details of their internalization pathway, we simultaneously added both micelle types with different fluorescent labels onto cells and evaluated their