Design and synthesis of peptide-coupled intracellular contrast agents for optical and MR imaging

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In recent years, many contrast agents have been developed and are being used as non-specific extracellular MR contrast agents. Another class is intracellular contrast agents whose prerequisite is to cross the plasma membrane by different mechanisms like endocytosis. For therapeutic applications, internalization is an absolute precondition, as the desired moiety has to reach its target within the cell. Cell penetrating peptides (CPPs) have been used to facilitate the delivery of macromolecules into the cells both *in vitro* and *in vivo*. The transfection ability of D-octaarginines, L-octaarginines and stearyloctaarginines has already been studied; stearyl-D-octaarginines showed improved transfection efficiency (1). This versatile approach can be used for the internalization of contrast agents into the cell for bimodal optical and MR imaging.

As a part of our ongoing research on synthesis of intracellular contrast agents, we have synthesized D-octaarginines, L-octaarginines and stearyloctaarginines covalently attached to the well established contrast agent (Gd-DTPA) and a fluorescent moiety, useful for MR imaging as well as optical imaging.

Synthesis was performed on polystyrene based Wang resin, containing Fmoc-protected arginine.The synthesized peptide was coupled with lysine linked to FITC by its ε amino group and further conjugated with DTPA. In case of lipid modified octaarginines stearyl-lysine was additionally introduced in between polyarginine and contrast agent. Finally complexation with Gd (III) was performed at pH 5-6 in room temperature. Comparative studies on the uptake mechanism were done by both optical (fluorescent microscopy and spectroscopy) and MR imaging techniques. The optimization of the cellular uptake can prove useful by coupling to the arginines and its derivative (stearyl) for the development of new intracellular targeted contrast agents.

(1) Shiroh Futaki, *et al*, Bioconjugate Chem. 2001, 12, 1005-1011