

## Lipopeptides Possessing Tripeptide, not Dipeptide, Head Groups Show Efficient DNA and siRNA Delivery

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**Statement of Purpose:** Synthetic vectors for gene (DNA/siRNA) delivery have drawn vast interest in the past two decades. Although there have been numerous examples of their application *in vitro* and *in vivo*, even a few in clinical trials, relatively low transfection efficacy (compared to viral vectors) still remains a significant challenge. In our recent work, we investigated novel cationic lipid vectors with a di- or tri- peptide as the head group, which differs from other commonly used amines or quaternary ammoniums. Although there are some examples employing peptides (mostly  $\geq 6$  amino acids) as vectors or part of a vector, they usually are expensive to produce, have limited transfection activity and show some cytotoxicity. The advantages of using these peptide head groups include ease of synthesis, improved solubility, good biocompatibility, and most importantly, good transfection efficacy *in vitro*. Among these lipopeptides, the vectors that possess a tripeptide head group show high transfection efficiency in multiple cell lines. We also explore the effect of different tripeptide sequences on transfection sequences.

**Methods:** The lipopeptides with di- or tri- peptide head groups were readily synthesized using common peptide and coupling chemistry. Several techniques were used to characterize their physicochemical properties and interactions with nucleic acids: nucleic acid binding affinity was measured by an ethidium bromide (EtBr) fluorescence quenching assay; size of the lipoplexes was obtained by dynamic light scattering (DLS); phase behavior was studied by differential scanning calorimetry (DSC) and X-ray diffraction. DNA transfection was carried out using a reporter gene beta-galactosidase assay system with CHO (Chinese Hamster Ovary), NIH3T3 (mouse fibroblast) and HUVEC (Human Umbilical Vein Endothelial Cells) cell lines. siRNA transfection was performed by knocking down glyceraldehyde 3-phosphate dehydrogenase (GAPDH) expression in HepG2 (human hepatocellular carcinoma) and A549 (human lung carcinoma) cell lines or luciferase in MDA-MB-231-luc (human breast adenocarcinoma) cell line.

**Results:** Lipopeptides with di- or tri- peptide head groups were synthesized and characterized. Interestingly, only those possessing a tripeptide, in which a cationic amino acid is separated from the hydrophobic domain by two other amino acids, show transfection activities. All the lipopeptides show similar binding affinity to DNA and form lipoplexes 200~300 nm in size. X-ray diffraction patterns show that the lipopeptides form lamellar structures both in the presence and absence of DNA. However, in the case of dipeptide head groups, the repeating period increases significantly upon adding DNA, which is not seen in tripeptide-containing lipoplexes. This observation implies a significant structural difference in the lipoplexes, whereby DNA is located inside the bilayers in the former, but located between adjacent bilayers in the latter. Furthermore, the

transfection activities of the lipopeptides possessing a tripeptide head group promoted us to explore the use of RGD, a well-known ligand for cell surface integrins, as the nucleic acid binding domain. These lipopeptide shows high transfection efficiency and minimum cytotoxicity in multiple cell lines. In some cell lines, they perform better than commonly used benchmarks Lipofectamine<sup>TM</sup>2000 (Invitrogen, Carlsbad CA) and siPORT<sup>TM</sup> NeoFX (Ambion, Austin TX).

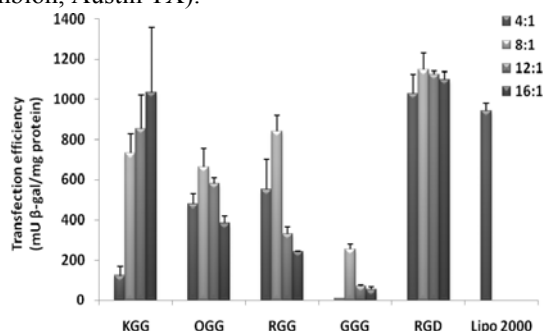


Figure 1. DNA transfection by lipopeptides with different tripeptide head groups in CHO cells

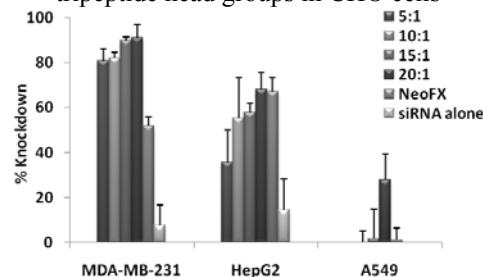


Figure 2. siRNA transfection by lipopeptide with RGD head group in three different cell lines

**Conclusions:** A series of novel lipopeptides possessing di- or tri- peptide head groups for gene delivery has been synthesized and characterized. Among them, only those with tripeptide headgroups show gene transfection activities. Although they all form lamellar structures, DNA is located at different positions in the bilayers. Considering that they are very similar in other properties, such as DNA binding affinity, lipoplex size and phase behavior, this structural difference is probably an important reason for the difference in transfection activity. Further optimization of the peptide head group by using a RGD resulted in very high DNA and siRNA transfection efficiency in several cell lines *in vitro*. These results indicate that both the spacer effect and the cell surface targeting, play a important role in the resulting transfection activity. The results of ongoing *in vivo* experiments will give us greater insight into the transfection mechanism and further direction to optimize the lipopeptide structure.

### References:

Prata CA. Bioconjugate Chem. 2008;19:418-420