

**IN VITRO DELIVERY OF ANTISENSE OLIGONUCLEOTIDES**

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Oligonucleotides (ODNs) do not readily get access to their desired site (cytoplasm/nucleus) of action due to their high hydrophilicity and high molecular weight. Therefore enhancing components (carriers) are needed. Most widely used *in vitro* are cationic lipids (liposomes). Other systems include peptide conjugates or complexes, polymers and nanoparticles. We used cationic lipids and polymers, with or without membrane active components DOPE or JTS-1 peptide [1,2] to study the delivery (into cytosol/nucleus) of active antisense phosphorothioate ODNs (PS-ODNs) into D 407 (retinal pigment epithelial) and CV-1 (monkey kidney fibroblast) cell lines expressing luciferase (LUC) [3]. Antisense effect was established with a 15-mer PS-ODN (5'- TGG CGT CTT CCA TTT-3') targeted to the initiation codon of luciferase. Additionally, a D 407 cell line was used to study the effect (increase in LUC activity) of a 18-mer O-methyl PS oligoribonucleotide (5'-CCU CUU ACC UCA GUU ACA-3) that masks the incorrect splicing site (in nucleus) in cells expressing the mutated form of luciferase (pLUC705) [4]. Cationic polymers polylysines (PLL with MWs of 4000, 20000 and 200000), polyethyleneimines (PEI 25 and 800 kDas) and lipid based carriers DOTAP, DOTAP/Chol, DOTAP/DOPE, DOTAP/DOPE/Chol were tested in PS-ODN delivery into D 407. JTS-1 (negatively charged) was added to the positively charged complexes containing DOTAP, PEI or PLL. Additionally, the effects on activity of the complexation medium was established by using water, MES-HEPES buffer or cell culture medium (DMEM) in the case of CV-1 cells expressing luciferase. In transfections of mutated D 407 cells (splicing correction) MES-HEPES or 5 % glucose were used. In our studies lipid based carriers with a membrane active component (especially DOTAP/JTS-1) were the most effective in delivering active PS-ODN into D 407 and CV-1 cells in sufficient amounts to cause significant antisense effect. Additionally, JTS-1 peptide maintains the activity of the complex in 5 and 10 % FBS containing DMEM (net neutral complex). This may be useful when the cells used in transfection experiments are sensitive to serum-free medium or extensive washings. Splicing correction studies in D 407 cells indicated, that due to the higher sensitivity of the method, delivery with e.g. DOTAP without enhancing components can be readily detected in this system as opposed to the luciferase expressing D 407 cells. All polymers used were ineffective (even with JTS-1) in both systems. Although PEI is a widely used and efficient plasmid (DNA) transfection agent, too strong binding of PS-ODN to PEI may affect the release from the complex. This is in contrast to modified (for nuclease protection) PO-ODN, that seems to be released and to be active

[5]. As for the complexation medium effects, lipid based carriers with DOPE or JTS-1 are most effective when complexed in glucose or water, while in the case of e.g. PS-ODN/DOTAP complexes, the complexation medium is not so relevant. The reason may be due to very heterogeneous complexes (size, morphology) formed in the case of DOPE containing complexes, especially in DMEM. For DOTAP alone the changes are not so drastic [6]. Overall, the results suggest that lipid based carriers with a membrane active component, in this case DOPE or JTS-1 peptide, are required for efficient delivery of PS-ODNs, although the complexation medium, among other factors, does have great effect on activity.

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