Abstract

The transfer of exogenous DNA into the nucleus is an inefficient process and is considered as a major limiting step for the nonviral gene delivery. One of the strategies to improve nuclear uptake of DNA is taking advantage of the cellular nuclear import machinerie by coupling a peptide bearing a nuclear localization signal (NLS) to the DNA. The aim of the present work is to analyse the role of synthetic NLS-peptides non covalently bound to DNA-complexes during the transfection process.

Peptides containing a virus derived NLS-sequence fused to an oligolysin-DNA-bindung domain were designed. Subsequently, peptide/DNA-complexes were generated and used to transfect human tumor cells *in vitro*. The result showed a 1.5 to 2.5-fold NLS-sequence specific increase of reporter gene expression. The transfection efficiency was related both to the used NLS-sequence and the employed peptide to DNA charge ratio. In order to gain an insight into the transfection process the physicochemical properties of peptide/DNA-complexes were examined and compared to the transfection efficiency. As a result, the NLS-sequence was shown to influence the DNA condensation rate, the ζ-potential as well as the aggregation behavior of the resulting DNA-complexes. The transfection efficiency was mainly determined by the size and the compaction rate of the complexes. Moreover, transmission electron microscopy observations showed the effective internalization of micrometer-sized aggregates, thus confirming the physicochemial study. Therefore, this result demonstrates an unspecific effect of the NLS-peptide on the gene transfer process. However, this effect strongly depended on the particular NLS-sequence. A more thorough analysis of the data was performed and suggests the occurence of unspecific interactions within the DNA-complex due to the particular amino acid composition of the peptide. This has strong implications for the optimization of nonviral multicomponent systems which was also discussed. In particular, the aggregation tendency represents an additional hurdle for the *in vivo*-application in the frame of human gene therapy.

As a consequence, the ability to generate colloidal stable DNA-complexes became a major issue of the present work. A potential solution arose from recent advances in the field of nanobiotechnology. The layer-by-layer technique consists in the stepwise deposition of oppositely charged polyelectrolytes resulting in the formation of well defined multilayers systems. Therefore, the aim was to use this technique in order to achieve stabilization of DNA-

complexes by electrostatic repulsion. As a model system, preformed polyethyleneimine/plasmid-DNA-complexes were combinded to the synthetic polyanions polyvinyl sulfate (PVS) or polystyrene sulfonate (PSS) under salt-free conditions. The results showed the generation of negatively charged DNA-nanoparticles which were colloidal stable in physiological salt conditions. Moreover, intratumoral injection of particles into transplantation tumors of nude mice resulted in relatively high reporter gene expression without apparent toxicity. Therefore, this result represents a significant advance in the development of DNA-containing nanoparticles for nonviral gene delivery.

The following step was the incorporation of NLS-peptides. Using PVS as the polyanion, 100 nm-sized colloidal stable particles were generated. A ζ-potential lying between – 42 mV and – 62 mV was measured depending on the incorporated peptide. Following *in vitro*-transfection, no significant reporter gene expression could be found. This is in agreement with other published studies using negatively charged particles. The *in vivo* delivery of the particles would require the incorporation of additional functional elements. Therefore, the nanoparticle formation process was investigated using physicochemical and morphological approaches. The results showed a penetration of the PVS into the peptide/DNA-complex leading to the generation of scrambled interpolyelectrolyte complexes. This was in contradiction to the original model of a core-shell-structure of the particle. Additionally, the DNA-containing particles showed a strong sensitivity regarding variations in the salt concentration. This was attributed to the influence of the ionic strength to electrostatic interactions between the polyelectrolytes as well as to the flexibility of the DNA molecule. Consequently, the entire particle formation procedure was adapted to physiological salt conditions. Using PSS as the polyanion, this was achieved following a dramatic increase of the peptide to DNA charge ratio from 3.0 to 20 and the subsequent increase of the PSS to DNA weight ratio from 1.2 to 50. Moreover, these results suggest a structured assembly of the particle consisting in a peptide/DNA-core surrounded by a PSS-shell. As a consequence, this work may provide a basis for the development of multilayered virus-like particles for nonviral gene delivery.