KL 2 - Chitosans as Gene Delivery Vehicles

S.P. Strand, K.M. Vårum

NOBIPOL, Department of Biotechnology, Norwegian University of Science and Technology, 7491 Trondheim, Norway

During the last decade, chitosans have emerged as promising non-viral DNA delivery systems. In contrast to synthetic polycations and cationic lipids, representing the two main categories of non-viral gene delivery systems, chitosans are biodegradable and show low toxicity. Although different chitosans are capable of complexing DNA into nano-sized particles (polyplexes), the gene delivery performance of chitosans has been found to vary strongly with molecular parameters such as the fraction of acetylated units (FA) and the degree of polymerization (DP). In addition to chitosan structure, the efficiency of chitosans is critically dependent on a range of other factors, such as details of polyplex formation, transfection protocol as well as the type of cell line transfected. As different gene delivery applications and routes of administration may require different vector properties, tailoring of a chitosan gene delivery system may be achieved through controlling of the chitosan structure and polyplex properties.

In collaboration with the Department of Pharmacy, Uppsala University, we have performed a series of studies with the aim to develop chitosan-based gene delivery systems. Here we will review some of the results, highlighting the gradual development from conventional high molecular weight samples to highly defined oligomers bearing targeting moieties. Initially, structure-function relationship was established for conventional high molecular weight samples. The transfection efficiency of chitosan polymers was found to be dependent on FA. Although transgene expression was observed after intratracheal administration to mice in vivo, the efficiency of chitosans was substantially lower than that of the golden standard, polyethylenimine. To overcome several drawbacks of high molecular weight chitosans including low solubility, high viscosity, high polyplex stability and formation of aggregated polyplexes, a series of low molecular weight chitosans was investigated. It was found that polyplexes based on well-defined oligomers with a number-average degree of polymerization (DPn) of 18 and 25 were superior to high molecular weight chitosans, showing both higher transgene expression and faster onset of action. Surprisingly, when fractionated samples of low polydispersity were used, it was found that the most efficient chitosan after in vivo administration to mouse lung showed poor performance in vitro and vice versa.

In a next step towards more efficient gene delivery, previously optimized chitosan oligomers were substituted with a GlcNAc-containing oligosaccharide with the aim to target membrane-bound lectins. The substitution resulted in increased cellular uptake and higher transgene expression as well as increase in the colloidal stability of polyplexes. The transfection efficiency of substituted chitosans was dependent on the DP of the backbone and the degree of substitution of the oligosaccharide branches, indicating the importance of a fine-tuned balance between polyplex stability and dissociation. The physical and colloidal stability of polyplexes were then further improved by using branched chitosans.