HIGH EFFICIENCY GENE TRANSFER USING CHITOSAN/DNA NANOPARTICLES WITH SPECIFIC COMBINATIONS OF MOLECULAR WEIGHT AND DEGREE OF DEACETYLATION

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Key Words : gene delivery, nanoparticles, plasmid DNA

Chitosan has shown potential for gene delivery [1,2], although the ideal molecular weight (MW) and degree of deacetylation (DDA) for this application have not been elucidated. To examine the influence of these parameters on gene delivery, we produced chitosans with different DDAs (98%, 92%, 80% and 72%) and depolymerized them with nitrous acid to obtain different MWs (150, 80, 40 and 10 kDa). We produced 64 formulations of chitosan/DNA nanoparticles (16 chitosans, 2 amine-phosphate (N:P) ratios of 5:1 and 10:1 and 2 transfection media pH of 6.5 and 7.1), characterized them for size and surface charge, and tested them for gene transfection in HEK 293 cells in vitro. Also, we investigated the binding of chitosan to DNA as a function of ionic strength, pH, buffer composition and chitosan molecular characteristics, as well as stability of the chitosan-plasmid DNA complexes. Several formulations produced high levels of transgene expression while two conditions, 92–10–5 and 80–10–10 [DDA–MW–N:P ratio] at pH 6.5, showed equivalence to our best positive control. The results also revealed an important coupling between DDA and MW of chitosan in determining transgene expression. Maximum expression was obtained with a certain combination of DDA and MW that depended on N:P ratio and the pH, but similar expression levels could be achieved by simultaneously lowering MW and increasing DDA or lowering DDA and increasing MW. Using isothermal titration microcalorimetry (ITC), we demonstrated that the binding constant and the entropy of interaction decrease with increasing pH and ionic strength, however, binding energetics and affinity of chitosan to DNA were not significantly affected by MW and DDA. In a stability test where nanoparticles were challenged by the presence of competing polyanions such as hyaluronan, chondroitin sulfate and heparin, only the latter was found to dissociate significantly the chitosan/DNA nanoparticles. As with gene expression, the stability of the nanoparticles depended on the MW and DDA of chitosan [3], suggesting a predominant role of particle stability, through co-operative electrostatic binding, in determining transfection efficiency. *[1] Koping-Hoggard et al., Gene Ther, 11:1441-52, 2004; [2] Huang et al., J Control Release, 106:391-406, 2005; [3] Strand et al., Biomacromolecules, 6:3357-66, 2005.*