**157b Synthesis and in Vitro Characterization of a Triblock Copolymer Carrier for Sirna**

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The ability to specifically down-regulate gene expression using the RNAi pathway in mammalian cells has tremendous potential as a therapeutic and as a tool in basic science. However, a delivery system capable of efficiently and specifically delivering siRNA to target cells is currently not available. Cationic polymers are some of the most widely studied and commonly utilized gene delivery vehicles, which can self-assemble with nucleic acids through electrostatic interactions. Limitations of cationic polymer based gene delivery systems include toxicity, aggregation, and unpacking of the nucleic acid inside the cell. We have designed a novel delivery system based on an ABC triblock copolymer in which each block is essential for siRNA binding and complex stability. The central hypothesis underlying our approach is that the self-assembling triblock copolymer of poly(ethylene glycol) (PEG), poly(propylene sulfide) (PPS) and a positively charged peptide (PEG-PPS-peptide)) can be tailored to complex siRNA via electrostatic and hydrophilic/hydrophobic interactions. Furthermore, the triblock polymer was designed to be reduction sensitive in order to take advantage of chemical differences in the cell organelles and cellular pathways to achieve efficient delivery of siRNA to the cytosol. The diblock polymer PEG45-PPS was synthesized using anionic polymerization of propylene sulfide. The peptide domain was bound to the PEG45-PPS copolymer using a disulfide exchange reaction. Triblock polymers with different PPS lengths (PPS5 and PPS10) were synthesized to study the effect of hydrophobic character of the delivery system on complex formation, complex size and transfection efficiency. The peptides chosen were the TAT peptide domain of HIV and polylysine (pK). The resulting triblock copolymers (PEG45-PPS5-TAT, PEG45-PPS10-TAT, PEG45-PPS5-pK and PEG45-PPS10-pK) were able to self-assemble with siRNA as shown by dynamic light scattering size measurements and gel electrophoresis. Complex size was found to be dependent on the amount of polymer used (charge ratio) and the length of the hydrophobic block (PPS). For both of the peptides tested and for triblock polymers with a small PPS block the size of the particles formed decreased as the amount of polymer (charge ratio) increased, with sizes of 200nm or less for the triblock copolymers. However, for the delivery system where the PPS unit was longer the trend was reversed, increasing the polymer content increased the size of the particles, with sizes of 600nm or less. These results show that the size of the particles that form can be tailored via electrostatic and hydrophobic interactions. Cell internalization studies analyzed via fluorescence microscopy and FACS showed that the triblock copolymers are able to transport siRNA inside the cell and that the internalization efficiency is higher from that of the peptides alone. siRNA transfection experiments in Hela cells have shown that the triblock copolymer PEG-PPS5-TAT is able deliver siRNA to the cytosol and down regulate the lamin AC gene. In contrast, using the TAT peptide as the delivery vehicle does not mediate gene down regulation. These studies have focused on the synthesis and in vitro characterization of a novel class of delivery system for siRNA based on self-assembling ABC triblock copolymers that bind to siRNA based on charge and hydrophilic/hydrophobic interactions.