Synthesis of Biodegradable Nucleic Acid Carriers by RAFT Copolymerization of Cationic Peptides with HPMA

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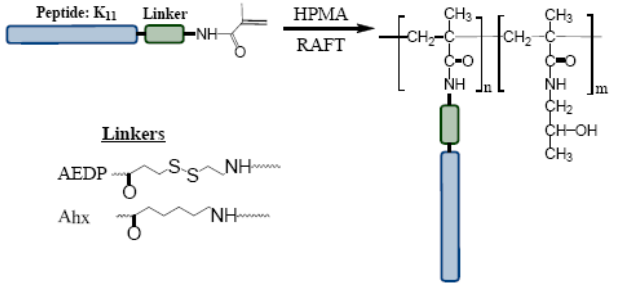
Statement of Purpose: Successful gene delivery is a multistep process that includes serum stability, cellular uptake, endosomal release and nuclear delivery. Synthetic gene carriers possessing multifunctional components may be more effective than single component systems at facilitating delivery through each of these steps. Generally, bioactivity and biocompatibility of peptides make them attractive modular components of a gene delivery system; however, efficient and well-defined integration of peptides into soluble polymeric-materials can be difficult. Recently, living polymerization techniques have enabled well-defined and narrowly disperse materials to be produced. This study focuses on development of N-(2-hydroxypropyl)methacrylamide (HPMA)-based copolymers that have incorporated cationic peptides for the delivery of nucleic acids for gene therapy.

Scheme 1. RAFT Copolymerization of HPMA-Peptide Copolymers



Methods: Functionalized cationic oligopeptides such as oligolysine (K_{11}) were produced by solid phase peptide synthesis (SPPS) using standard Fmoc chemistry. The synthetic strategy incorporated either a non-reducible or a reducible linker into the peptide backbone which was then capped on the amino-terminus with either acrylamido or methacrylamido functional groups. The cationic peptides were then copolymerized with HPMA by RAFT polymerization. All polymerizations were done in aqueous acetate buffer. In order to determine optimal polymerization conditions, the ratio of initial HPMA monomer to initial chain transfer agent (M_o/CTA_o) and the ratio of CTA_o to initial initator (CTA_o/I_o) were varied. Conversion of monomers to polymers was followed using NMR. Characterization of polymers was done by SEC analysis in-line with a miniDAWN TREOS light scattering detector (Wyatt) and a Optilab rEX refractive index detector (Wyatt). The peptide component was quantified by ninhydrin assay and with amino acid analysis. HPMA-peptide copolymers were evaluated for DNA condensation and cell transfection abilities by YOYO-1 quenching assay and luciferase assay, respectively.

Results: Initial polymerization experiments demonstrated that RAFT polymerization of cationic peptides with HPMA resulted in homogenous incorporation of cationic peptides into the final copolymer (see Figure 1). In contrast, higher-molecular weight peptide monomers were incorporated only after the monomer had become significantly depleted. This produced populations of HPMA homopolymers and gradient copolymers. Initial RAFT-polymerization of HPMA-peptide copolymers produced broadly disperse copolymers (Pd ranged from 1.6 to 3.0). By reducing the initiator (I_o), the polydispersity was reduced (Pd of 1.3). Following optimization, acrylamido-functionalized peptides were homogenously incorporated into statistical HPMA copolymers by RAFT polymerization. Conversion of monomers to polymers as determined by NMR demonstrated that nearly all of the monomers were consumed in 48 hours. All K_{11}-HPMA copolymers efficiently condensed DNA into nanoparticles. The nanoparticles were shown to have stability under physiological salt concentrations due to hydrophilic HPMA. Reducible copolymers showed less toxicity to cultured cells but also lower transfection efficiency. Results will be presented.

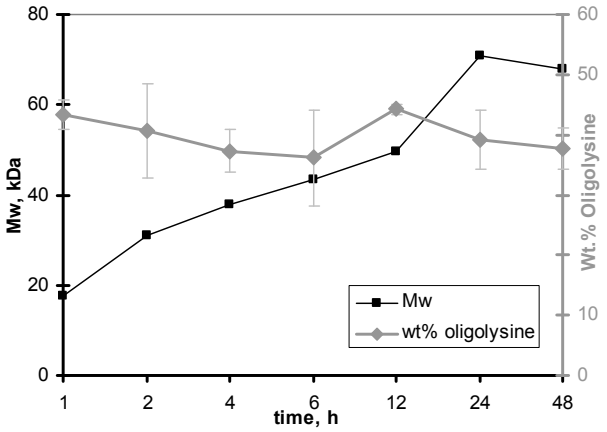


Figure 1. Molecular weight evolution and incorporation of oligolysine peptides into HPMA copolymers.

Conclusions: Modular approaches for gene therapy and drug delivery have been well established. However, pharmacological use has been limited by the diverse products produced. By employing RAFT polymerization, narrowly-disperse and well-characterized HPMA-oligopeptide copolymers were produced. Inclusion of the disulfide element allowed the cationic component of the peptide to degrade into smaller elements that have diminished cellular toxicity. Furthermore, the polymeric backbone can be engineered to be below the renal threshold allowing it to be eliminated from a biological system. Another promising aspect of this study is the inclusion of more diverse peptides into copolymers through RAFT polymerization that could lead to modular, multifunctional constructs capable of delivery of nucleic acids to targeted cells and transport from endosomes to the nucleus.

References:

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Aknowledgments:

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