Mechanistic Insight into Statistical HPMA-oligolysine Copolymers for Gene Delivery

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ABSTRACT SUMMARY

Peptide-based polymers are promising vehicles for gene delivery due to the ability to incorporate peptides to specifically address various intracellular barriers, such as cellular uptake and endosomal escape. Recently, our group optimized a panel of statistical HPMA-oligolysine copolymers to investigate variations in oligolysine length and polymer molecular weight. We showed that comb-like polymers synthesized using K\textsubscript{10} (10-mers of lysines) were efficient at gene delivery whereas comparable polymers synthesized using K\textsubscript{15} performed poorly with delivery efficiencies similar to poly(L)lysine. The goal of this study was to elucidate differences in transfection mechanisms between these two polymers in order to better identify key parameters that affect gene delivery. We demonstrate that polyplex morphology, unpackaging, and cellular uptake are important properties to consider when designing polymers for gene delivery. Specifically, polyplexes with smaller aspect ratios, the ability to unpack more easily, and high uptake efficiency correlated with higher in vitro transfection efficiencies in our HPMA-oligolysine system.

INTRODUCTION

Successful gene delivery requires vectors capable of overcoming several extracellular and intracellular barriers, such as cellular uptake, endosomal escape, effective unpackaging of therapeutic cargo, and nuclear import. Peptide-based polymers have been used as nucleic acid carriers to tackle these barriers by the incorporation of specific peptides for cell targeting, endosomal-lytic activity, and nuclear localization sequences, to name a few. These peptides can also be incorporated into well-defined narrowly-disperse statistical polymers, synthesized using living radical polymerization techniques, such as reversible-addition-fragmentation polymerization (RAFT).

Previously, our group reported the synthesis and characterization of a series of HPMA-oligolysine statistical copolymers for the delivery of plasmid DNA, optimizing oligolysine peptide length and polymer molecular weight\textsuperscript{1}. We found that a peptide length of ten lysines (K\textsubscript{10}) and a degree of polymerization of 150 (DP 150) performed almost as well as 25 kDa branched polyethylenimine (PEI), despite a lack of incorporated endosomal escape capabilities. Interestingly, a polymer with a 50% longer oligolysine length (K\textsubscript{15}) was inefficient at gene transfer.

The goal of this work is to identify key differences between these two similar polymers that might affect transfection efficiency. These studies demonstrate that differences in polyplex morphology, unpackaging, and cellular uptake may lead to the increased transfection efficiency of pHK10. In addition, pHK10 and pHK15 polyplexes were taken up more efficiently in GAG-deficient cells, indicating possible extracellular inhibition of oligolysine-containing polyplexes.

EXPERIMENTAL METHODS

Synthesis of peptide monomers, methacrylated AhxK\textsubscript{10} (MaAhxK\textsubscript{10}) and methacrylated AhxK\textsubscript{15} (MaAhxK\textsubscript{15}) were synthesized on a solid support of Rink amide following standard Fmoc/tBu chemistry on an automated PS3 peptide synthesizer and cleaved off the solid support with a standard acidic cleavage cocktail (TFA/TIPS/DMB) for 2 h while gently shaking. Peptides were then precipitated in cold ether and lyophilized. The monomers were analyzed using RP-HPLC and MALDI-TOF mass spectrometry, and were shown to have greater than 95% purity after cleavage. Copolymers of HPMA and either MaAhxK\textsubscript{10} or MaAhxK\textsubscript{15} were synthesized via RAFT polymerization under aqueous conditions, using ECT as the chain transfer agent. The resulting polymers were dialyzed, lyophilized, and characterized by size exclusion chromatography and amino acid analysis.

For uptake studies, luciferase-expressing plasmid DNA was radiolabeled using nick translation and 2‘-deoxyctydine 5‘-triphosphate, [5–H], according to manufacturer’s instructions. A mixture of unlabeled pDNA and [\textsuperscript{3}H]DNA was used to formulate polyplexes (N/P 5). HeLa cells were treated with radiolabeled polyplexes for up to 4 h in OptiMEM, and then chased for up to 20 h in complete media. At various time points, cells were washed with PBS, and incubated with CellScrub to remove membrane-bound polyplexes. Cells were then trypsinized and collected. All washes and cell samples were analyzed for radioactivity by scintillation counting (Beckman LS-6500).

For microscopy and unpackaging studies, polyplexes were formulated at a charge (N/P) ratio of 5. Polyplex morphology was determined by electron microscopy on a hydrophilic surface, rendered by glow discharge for 45 s. Polyplexes were applied to 400-mesh copper/formvar grids for 30 min. The grids were then rinsed in dH\textsubscript{2}O, and negatively-stained with 4% (w/v) uranyl acetate. Images were taken with a JOEL 1010 transmission electron microscope (Fred Hutch CRC). Polyplex unpackaging was determined by treating polyplexes with various amounts of heparan sulfate for 5 min at RT. The treated sample was then mixed with loading buffer and applied to a 0.8% agarose gel. DNA was visualized with ethidium bromide using a UV transilluminator.

RESULTS AND DISCUSSION

Differences in polyplex uptake may account for differences in transfection efficiency. To determine cellular uptake of polyplexes, HeLa cells were treated with radiolabeled polyplexes for up to 4 h,
and then chased for up to 20 h. Three lysine-based polymers were tested: poly(L)-lysine (PLL), pHK10 and pHK15. pHK10 polyplexes showed the most efficient uptake PLL, pHK15, and DNA (Figure 1). These results indicate that despite polyplex association with the cell, lack of efficient cellular uptake may lead to reduced transfection efficiencies.

Since a delay in polyplex unpackaging has been implicated in poor DNA release, and thus poor transfection efficiency, the unpackaging ability of each polymer was determined by competitive displacement with heparan sulfate (HS). Slight differences in polyplex unpackaging were seen between pHK10 and pHK15 formulations; pHK10 polyplexes were mostly unpackaged by 11 µg HS, while pHK15 polyplexes needed at least 14 µg HS to unpackage (Figure 3). However, neither formulation fully unpackaged, even with 22 µg HS. In contrast, PEI fully unpackaged at less than 1 µg HS. These results indicate that delayed polyplex unpackaging may lead to decreases in transfection efficiency.

CONCLUSION
In summary, HPMA-oligolysine copolymers of different peptide lengths (K_{10} and K_{15}) show differences in polyplex morphology, unpackaging ability, and cellular uptake that may lead to differences in transfection efficiency. pHK10 polyplexes formed particles with smaller aspect ratios that unpackaged more easily and were taken up more efficiently than pHK15 polyplexes. Thus, these parameters are important to review when designing materials for gene delivery.

REFERENCES

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