Evaluation of generations 0 and 1 dendrimer conjugated bio-reducible polymer (PAM-ABP) for efficient gene delivery

Kihoon Nam, Sung Wan Kim

Center for Controlled Chemical Delivery, Department of Pharmaceutics and Pharmaceutical Chemistry, University of Utah, Salt Lake City, UT 84112, USA
Kihoon.Nam@utah.edu

ABSTRACT SUMMARY
We synthesized and investigated dendrimer conjugated bio-reducible polymer (PAM-ABP) generation 0 and 1. The newly synthesized PAM-ABPs form more compact polyplexes with plasmid DNA at lower weight ratios compared to ABP. In addition, the PAM-ABP maintains more stable polyplexes in a reductive environment. These stable polyplexes showed enhanced cellular uptake and increased transfection efficiency compared to ABP and branched PEI 25 kDa.

INTRODUCTION
Gene therapy offers the potential to treat human diseases using therapeutic gene. The safe and effective gene delivery system is the most significant challenge for successful gene therapy. Generally viral or non-viral vectors are used to deliver a therapeutic gene. Non-viral vector systems usually make use of either naked pDNA only or various kinds of gene carriers such as lipids, synthetic polymers and peptides. Compared to viral vectors, non-viral gene carriers have the advantages of large-scale production, non-immunogenicity, flexible gene loading capacity. The gene carriers with high transfection efficiency and low cytotoxicity are most important part of the non-viral vector systems.

The introduction of arginine can increase the cell-penetrating ability of polymeric gene carriers. Various arginine conjugated polymers have been developed and showed high transfection efficiency. Arginine-grafted poly (cystaminebisacrylamide-diaminohexane) (ABP) also demonstrated in vitro to have a high transfection efficiency and low cytotoxicity. However, to optimize transfection efficiency through compact polyplex formation, the weight ratio of ABP had to be increased to above 20. This higher weight ratio limits the use of ABP in vivo.

To overcome the limitations of the low molecular weight ABP in vivo, we synthesized and investigated PAM-ABP generations 0 and 1. In this study, we describe: 1) the synthesis and characterization; 2) the stability; 3) extent of cellular uptake; 4) transfection efficiency and cytotoxicity profile of PAM-ABP.

EXPERIMENTAL METHODS
Arginine-grafted poly (cystaminebisacrylamide-diaminohexane), ABP, was synthesized as previously reported. ABP was dissolved in 0.1M phosphate buffered saline (PBS, pH 7.2, 0.15 M NaCl). A 1.2 equivalents of SPDP dissolved in DMF was added to the ABP solution and stirred for 1 h. And then, the mixture was dialyzed and lyophilized. For the thiolation of PAMAM, PAMAM was dissolved in 0.1 M PBS with 2mM EDTA (pH 8.0). Two equivalents of Traut’s reagent per surface primary amines of PAMAM G0 and G1 were added to the PAMAM solution with stirring, and the mixture was further reacted for 2 h. And then, the product was purified from excess Traut’s reagent using a sephadex G-10 column and lyophilized. Then, PAMAM-SH (1.0 equiv.) and ABP-SPDP (1.2 equiv.) were dissolved in 50 mM PBS with 10 mM EDTA (pH 7.2) and the reaction mixture was stirred for 4h. And then, the mixture was dialyzed and lyophilized.

The particle size and zeta-potential values of the polyplexes were measured using a Nano ZS at 25 °C.

The stability of the PAM-ABP polyplex under reductive condition was determined by agarose gel electrophoresis. In order to compare the degree of DNA release, each polyplex was incubated in the presence of 5 mM DTT for 30min at 37 °C.

The cellular uptake of polyplexes into A549 cells was investigated by fluorescence activated cell sorting (FACS) using YOYO-1 labeled plasmid DNA. Polyplexes were prepared with 0.5 μg of YOYO-1 labeled plasmid DNA in FBS-free medium, and the mixtures were incubated for 30 min at room temperature. The polyplexes were added to the cells and incubated for 4h at 37°C in serum-free medium. Then, medium was removed and cells were washed two times with ice-cold DPBS. After trypsinization, the cells were collected by centrifugation and resuspended in 500 μL of 1% FBS in ice-cold DPBS. The degree of cellular uptake was examined by using the BD FACScan analyzer.

The transfection efficiency of PAM-ABP was compared to the transfection efficiency of ABP in A549 cells using firefly luciferase and green fluorescent protein (GFP) expression.

The cytotoxicity of the polymers was measured by MTT assay.

RESULTS AND DISCUSSION
Arginine-grafted bio-reducible poly(disulfide amine) (ABP) was incorporated into the poly(amido amine) (PAMAM) dendrimer, creating a high molecular weight bio-reducible polymer, PAM-ABP, to overcome the limitations of the low molecular weight. Based on the results of the 1H NMR, four ABPs had been conjugated to the four primary amines of PAMAM G0 and eight ABPs.
had been conjugated to the eight primary amines of PAMAM G1.

Figure 1. The structure of PAM-ABP G0 and G1

Zeta-potential values and particle sizes of the ABP, PAM-ABP G0 and G1 polyplexes were measured to determine the details of complex formation. The PAM-ABP G1 forms more compact polyplexes than ABP and PAM-ABP G0.

<table>
<thead>
<tr>
<th></th>
<th>Size (nm)</th>
<th>Zeta potential (mV)</th>
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<tbody>
<tr>
<td>ABP</td>
<td>132.9</td>
<td>25.3</td>
</tr>
<tr>
<td>PAM-ABP 1</td>
<td>116.1</td>
<td>18.1</td>
</tr>
<tr>
<td>PAM-ABP 0</td>
<td>110.6</td>
<td>24.6</td>
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Table 1. Zeta-potential values and particle sizes

In order to determine the stability of polyplexes, the pDNA release from the polyplexes in the presence of DTT at 37 °C was determined by agarose gel electrophoresis. The PAM-ABP G1 forms more stable polyplexes than ABP and PAM-ABP G0.

The cellular uptake of the PAM-ABP with YOYO-1 labeled pDNA was determined by flow cytometer. At a weight ratio of 5, PAM-ABP G0 (76.20%) and G1 (83.13%) showed higher cellular uptake than the ABP polyplexes (16.39%).

The transfection efficiency of PAM-ABP G1 was compared to the transfection efficiency of bPEI 25kDa. ABP and PAM-ABP G0 in A549 cells. PAM-ABP G1 showed higher transfection efficiency than ABP, PAM-ABP G0 and bPEI 25kDa.

CONCLUSION

In this study, we describe the synthesis of PAM-ABP generation 0 and 1. And, the potential of PAM-ABP was evaluated by particle size, surface charge, stability, cellular uptake and transfection efficiency. Based on these results, dendrimer conjugated bioreducible polymer, PAM-ABP, has superior efficacy compared to low molecular weight ABP and therefore may be a promising carrier for clinical gene therapy.

REFERENCES

ACKNOWLEDGMENTS

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