Chiral cationic polyamines for chiral microcapsules and siRNA delivery

Justin Gharavi a, Patrick Marks a, Kelly Moran a, Brett Kingsborough a, Ruchi Verma b, Yuan Chen b, Ruitang Deng b, Mindy Levine a, a

a Department of Chemistry, University of Rhode Island, 51 Lower College Road, Kingston, RI 02881, United States
b College of Pharmacy, University of Rhode Island, 7 Greenhouse Road, Kingston, RI 02881, United States

A R T I C L E   I N F O

Article history:
Received 25 June 2013
Revised 16 August 2013
Accepted 19 August 2013
Available online 26 August 2013

Keywords:
Polyamine
Chirality
siRNA
Transfection

A B S T R A C T

Reported herein is the use of chiral cationic polyamines for two intriguing applications: fabrication of chiral covalently-linked microcapsules, and enantiospecific delivery of siRNA to Huh 7 cells. The microcapsules are easily fabricated from homochiral polymers, and the resulting architectures can be used for supramolecular chiral catalysis and many other potential applications. Enantiospecific delivery of siRNA to Huh 7 cells is seen by one ã enantiomerã of the polymers delivering siRNA with significantly improved transfection efficiency and reduced toxicity compared to the ã enantiomericã polymer and commercially available transfection reagents. Taken together, the use of these easily accessible polyamine structures for diverse applications is highlighted in this Letter herein and can lead to numerous future research efforts.

Polyethyleneimines (PEIs) are a well-studied class of polymers.1-2 These polymers are synthesized commercially via the ring opening of aziridine (Scheme 1, Reaction 2),3-5 although this process leads to highly branched polymers6 with significant polydispersity indexes. The controlled synthesis of linear PEIs occurs via the cationic ring opening of oxazolines,7-9 followed by hydrolysis of the resulting formamides (Scheme 1, Reaction 1). Using chiral oxazolines as substrates for the polymerization reaction provides straightforward access to homochiral PEIs,10-12 with chiral centers at every polymer repeat unit.

The significant interest in PEIs is driven largely by various applications of PEIs in fields including chiral catalysis,13-16 drug delivery,17-18 and oligonucleotide complexation and delivery.19-20 PEIs have also been covalently linked to form PEI-derived microcapsules,21 which have been used for site-isolated catalysis.22,23 In one example, the Lewis basic PEI catalyzed a reaction in the same reaction vessel as a Lewis acidic nickel catalyst, which was used to catalyze the second reaction.24,25

Use of the same PEI scaffold for multiple applications has rarely been reported, although such multi-purpose polymers would have significant operational advantages. Reported herein is the use of a single PEI scaffold for two purposes: the fabrication of covalently-linked chiral microcapsules, and the efficient delivery of siRNA to Huh7 cells.26

The chiral PEIs were synthesized via the cationic polymerization of 4-benzyl-2-oxazoline (1a) (both R and S configurations), followed by the hydrolysis of the initially formed polyformamide (Scheme 2). The resulting polymers were characterized by 1H NMR spectroscopy, and the results were in agreement with literature-reported spectra.11 Using this methodology, polymers with 13 and 30 repeat units were formed, with both R and S configured side chains.

Once synthesized, the homochiral PEIs were cross-linked to form homochiral microcapsules following the procedure developed by McQuade and co-workers.22 Briefly, polymers 2a were dissolved in methanol, and added to a solution of 2% Span 85,

Scheme 1. General synthetic methods for linear and branched polyethyleneimine (PEI).
followed by the addition of 2,4-tolylene diisocyanate (TDI, compound 7) (Equation 1), which cross linked the microcapsules to form a polyurea coating. The resulting polyurethane-type structures have been shown to be stable in a variety of aqueous media. After thorough solvent evaporation, chiral microcapsules were obtained.

The resulting microcapsules were imaged using transmission electron microscopy (TEM), and some images are shown in Figure 1. The diameters of the particles ranged from 57 to 250 nm, with an average diameter of 141 nm (± 35 nm; 62 particles measured). These new supramolecular architectures contain narrow size distributions and uniform structures, in good agreement with literature-reported results for achiral microcapsule analogs.

The newly formed microcapsules contain a variety of features that make them particularly amenable to supramolecular chiral catalysis, including: (a) multiple chiral centers, covalently confined in a small space; (b) multiple amino groups that can be protonated or deprotonated over a wide pH range; and (c) a hydrophobic core resulting from the hydrophobic benzyl side chains.

To investigate the effect of capsule formation on the resulting supramolecular chiral environment, the newly synthesized chiral microcapsules were used as catalysts for the transamination reaction of ketoacids to amino acids (Equation 2). Obtaining good enantioselectivities in such transamination reactions has been an ongoing research problem. Preliminary results indicate that the microcapsule-catalyzed reactions proceeded with significantly higher enantioselectivities compared to the polymer-catalyzed reactions (up to 20% enantiomeric excess (ee) obtained for the synthesis of l-valine, under conditions where the polymer itself yielded 4% ee).

Efforts to optimize the reaction conditions are in progress. Interestingly, the chiral PEIs also functioned as efficient siRNA delivery agents. Although there are many reported examples of PEIs used for siRNA and DNA delivery, many of these delivery vehicles suffer from high cytotoxicity. The development of gene delivery agents that are both effective and less toxic remains a highly relevant research objective.

The following 4 polymers were investigated as potential siRNA delivery agents: R-2a-13; S-2a-13; R-6-13; and S-6-13, where the R/S designation refers to the chirality of the side chain and the number 13 refers to the number of repeat units in the polymers. The efficacy of these polymers in transfecting an Alexa488-labeled control siRNA sequence to Huh7 cells was measured by determining the intracellular fluorescence 24 h post-transfection. The results obtained using the chiral polyamines were compared to results obtained using commercially available transfection reagents: Genjet siRNA Transfection Reagent (SignaGen Laboratories); HiPerFect Transfection Reagent (Qiagen Laboratories); and Lipofectamine 2000 (Invitrogen Technologies).

Figure 2 shows a graph of the intracellular fluorescence of Huh7 cells following their incubation with Alexa-labeled siRNA with various delivery reagents. The intracellular fluorescence obtained with compounds S-6-13 and S-2a-13 is substantially higher than the fluorescence observed with positive controls Lipofectamine and Genjet, indicating the polymers’ ability to transfect siRNA efficiently (Table 1). More interestingly, compounds R-2a-13 and R-6-13, which are identical except for the three-dimensional
40 After 24 h of incubation, the absorbance of the cells reduced to 89%, and compounds S-6-13 reduced it to 82%. By comparison, Lipofectamine performed better than either the S enantiomer or the racemic DOTAP mixture.

Figure 2. Chart of the intracellular fluorescence of Huh7 cells after transfection with siRNA (all PEIs were used at a 1000 nM final concentration).

Table 1
Transfection efficiencies of chiral PEIs and commercial transfection agents

<table>
<thead>
<tr>
<th>Transfection agent</th>
<th>Intracellular fluorescence (normalized to 1.00 for cells alone)</th>
</tr>
</thead>
<tbody>
<tr>
<td>S-6-13</td>
<td>1.26</td>
</tr>
<tr>
<td>S-2a-13</td>
<td>1.30</td>
</tr>
<tr>
<td>Lipofectamine</td>
<td>1.05</td>
</tr>
<tr>
<td>GenJet</td>
<td>1.04</td>
</tr>
<tr>
<td>R-2a-13</td>
<td>1.06</td>
</tr>
<tr>
<td>R-6-13</td>
<td>1.05</td>
</tr>
</tbody>
</table>

dichroism spectroscopy. These differences in chirality affect the polymers' solubility and their interactions with DNA, and as shown here, their transfection efficiencies.

In summary, chiral polymers 6 and 2a were synthesized via straightforward, well-precedented procedures. These polymers were used for two novel applications: the fabrication of chiral, covalently-linked microcapsules, and the transfection of siRNA to Huh7 cells. The chiral microcapsules can be used for a number of potential applications in supramolecular chiral catalysis and in supramolecular enantiomer separations. The chirality-dependent siRNA transfection also provides an intriguing platform for further investigation. In particular, polymer S-2a-13 demonstrated good transfection efficiency and limited toxicity, and will be used for further biochemical investigations. The results of these and other experiments will be reported in due course.

Acknowledgments

This work was supported by an Institutional Development Award (IDeA) from the National Institute of General Medical Sciences of the National Institutes of Health under grant number 8 P20 GM103430-12 to M.L. R.D. is supported by the National Institutes of Health (NIH) (Grant No. R01DK087755).

Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2013.08.083.

References and notes