Organic & Biomolecular Chemistry

PAPER

Cite this: *Org. Biomol. Chem.*, 2015, **13**, 9445

Experimental design, modeling and optimization of polyplex formation between DNA oligonucleotides and branched polyethylenimine†

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Received 16th June 2015,
Accepted 29th July 2015

DOI: 10.1039/c5ob01189b

www.rsc.org/obc

The complexes formed by DNA and polycations have received great attention owing to their potential application in gene therapy. In this study, the binding efficiency between double-stranded oligonucleotides (dsDNA) and branched polyethylenimine (B-PEI) has been quantified by processing of the images captured from the gel electrophoresis assays. The central composite experimental design has been employed to investigate the effects of controllable factors on the binding efficiency. On the basis of experimental data and the response surface methodology, a multivariate regression model has been constructed and statistically validated. The model has enabled us to predict the binding efficiency depending on experimental factors, such as concentrations of dsDNA and B-PEI as well as the initial pH of solution. The optimization of the binding process has been performed using simplex and gradient methods. The optimal conditions determined for polyplex formation have yielded a maximal binding efficiency close to 100%. In order to reveal the mechanism of complex formation at the atomic-scale, a molecular dynamic simulation has been carried out. According to the computation results, B-PEI amine hydrogen atoms have interacted with oxygen atoms from dsDNA phosphate groups. These interactions have led to the formation of hydrogen bonds between macromolecules, stabilizing the polyplex structure.

1. Introduction

Gene therapy, a method used to introduce genetic material into cells to treat various maladies, requires specific therapeutic genes and efficient, yet non-toxic gene delivery systems.[1] Cationic polymers as non-viral gene delivery systems (vectors) have great potential to create pharmaceuticals from nucleic acids.[2–4] They have less specific immune responses, and are generally safer and easy to design with more flexible structures and chemical properties for various purposes.[5–9] Among the available cationic polymers, polyethylenimine (PEI), including branched (B-PEI) and linear polyethylenimine (L-PEI), is the most effective vector for various types of polymeric gene carriers, and has thus been extensively used for both *in vitro* and *in vivo* gene delivery.[10–12] One of the most important properties of PEI is its high cationic charge density. The theoretical ratio of primary, secondary and tertiary amino groups in B-PEI has been calculated as 1 : 2 : 1, respectively,[13] and there is a close relationship between the pH of PEI and the positive charge density on PEI.[14] Previous reports have revealed that the PEI protonation degree at physiological pH is 20%.[8,15] Later, other authors[16,17] have determined by both experiments and computations that for linear, star-like and comb-like PEI, the degree of protonation of amine groups was about 50% at pH = 7.4. Recently, a cost-effective gene transfection by plasmid DNA compaction at pH = 4.0 using PEI has also been reported.[18]

Besides plasmid DNA, PEI has also been widely used for the compacting and delivery of short, 20–25 double-stranded nucleotides for siRNA-mediated or oligonucleotide gene therapy.[13,19–22] Although plasmid DNA-based polyplexes are well characterized, it was recently shown that not all knowledge can be adapted from DNA-based polyplexes to short oligonucleotide-based polyplexes, as the synthetic sequence is around 250 times smaller and shows a higher conformational rigidity. Thus, Wagner and co-workers[23] have reported a

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†Electronic supplementary information (ESI) available. See DOI: 10.1039/c5ob01189b