LINEAR AND BRANCHED POLYETHYLENIMINES
AS NONVIRAL VECTORS FOR GENE DELIVERY


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Introduction

Gene therapy, the delivery of DNA or RNA into cells, has gained momentum over the past few decades as a potent tool for treatment of genetic disorders and cancer. A significant obstacle to gene therapy is developing effective carriers that protect the DNA or RNA from serum nuclease degradation, facilitate cellular uptake, and enable transfer of the cargo into the nucleus or cytoplasm. Nonviral vector systems, based on cationic lipids, dendrimers, peptides, and polymers,[1] are currently preferred for gene delivery because they are much safer than viral systems,[2] which are burdened by immunogenic or inflammatory responses.

Polyethylenimine (PEI) is a cationic polymer widely adopted in nonviral gene delivery systems both *in vitro* and *in vivo* due to its high transfection efficiency.[3] PEI is capable of condensing plasmid DNA and RNA through electrostatic interaction to form complexes that are internalized into cells through endocytosis. This ability is attributed to the "proton sponge" characteristic of PEI, in which the single nitrogen per monomer subunit in PEI forms an ionic interaction with the phosphate backbone of nucleic acids. Thus, DNA or RNA complexes (or polyplexes) are readily formed through mixing with PEI.[4] These PEI complexes result in increased transfection efficiency due to the large buffering capacity of PEI, leading to changes in endosomal osmolarity, and resulting in lysis and release (endosomal escape). PEI-based vectors have been used to deliver oligonucleotides, plasmid DNA, as well as RNA and intact ribozymes.[5]

The molecular weight, structure, and branching of PEI can each influence the condensation behavior, complex size, and transfection efficiency of the vector. For example, the transfection efficiency of PEI increases by increasing the molecular weight of the polymer. Higher molecular weight PEI generally results in a decreased complex size. However, higher molecular weight PEI polymers also lead to higher toxicity.[1] The cytotoxicity of PEIs is the result of the high positive charge density within the polymer chains that can lead to destabilization of the cell wall and cellular necrosis. The overall cytotoxicity of the PEI vectors can be modulated by adjusting the nitrogen-to-phosphate (N/P) ratio of the nucleotide/PEI complex.[6] The preferred molecular weight for PEI for gene delivery is estimated to be in the range of 5,000–25,000 Da, whereas lower molecular weight PEI transfection efficiency can outperform the higher molecular weight counterparts only if higher N/P ratios are used.[7]

Branched PEIs possess higher charge density and carry approximately equal amounts of primary, secondary and tertiary amino groups. Linear PEI contains only secondary amino groups and therefore linear PEIs are somewhat less cytotoxic.[8] In general, primary amines condense DNA better than other amines due to their higher protonation. In addition, binding capacity is correlated to the number of primary amines, and complex stability increases with increasing primary amine content, leading to higher transfection efficiencies. Linear PEI is a particularly effective gene transfer agent. It has lower complexation capability than branched PEI toward nucleotides, but at the same time linear PEI is more efficient than branched PEI due to its topology and lower cytotoxicity as demonstrated in a number of *in vivo* studies.[9]

The design of the PEI polymeric carrier can be challenging since transfection efficiency and cytotoxicity depend on the physiochemcial properties of the polymer. In addition, PEI can be used for different gene therapy applications as a carrier for plasmids, oligonucleotides, or siRNA. In general, molecular weight and branching need to be adjusted depending on application to design stable complexes with the desired release rates.

Methods: PEI Solutions and Complexes

Linear PEI

Linear PEI is not directly soluble in water at ambient temperature. To make a PEI solution based on monomer units (7.5×10^{-3} M), linear PEI (0.323 g/L) is added to endotoxin-free de-ionized H_2O and stirred for approximately 1 h on low heat until all of the particles are in solution. The volume is then adjusted with de-ionized H_2O to achieve the desired concentration. Allow the solution to cool to room temperature, neutralize to the desired pH, and sterile filter (0.22 µM). A large PEI stock solution can be prepared and stored frozen at –20 °C. Aliquots of linear PEI can be thawed and stored at 4 °C while in use. Aliquots of linear PEI may be adjusted to pH 7.0, 8.0, and 9.0 for testing the effect of the pH of the PEI solution on the transfection.[10]