Freeze-drying Differentially Influences the Transfection Efficiency of Polyethylenimine-DNA Condensates

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Statement of Purpose:

Non-viral gene vectors have been increasingly utilized in various gene-based therapies due to safety concerns with viral gene vectors. A variety of cationic polyelectrolytes including polyethylenimine (PEI) have been used to enhance gene transfer by condensing plasmid DNA[1]. It is necessary to store the condensed plasmid DNA (pDNA) in a powder form for many applications. However, freeze-drying PEI-DNA complexes has been reported to greatly reduce gene expression levels when compared with transfecting with fresh PEI-DNA[2]. The mechanism behind this reduction is unclear. This study is based on the hypothesis that the decrease of gene expression is caused either by changes in complex size, complex zeta potential, or the strength of interaction between PEI and pDNA upon freeze-drying. The molecular structure of PEI was also hypothesized to regulate the effect of freeze-drying. In these studies, the association between pDNA and PEI following freeze-drying was monitored utilizing a fluorescent resonance energy transfer (FRET) technique.

Methods:

Plasmid DNA encoding luciferase was condensed with a branched (M_w ~ 25 kDa) or linear (M_w ~ 22 kDa) PEI while varying the charge ratio between PEI and pDNA. The pDNA complexes were frozen in liquid Nitrogen and freeze-dried overnight. Dried samples were then reconstituted in 1mL of PBS. MC3T3 cells plated on cell culture plates were incubated in αMEM supplemented with FBS and PS for 24 hours. Then, the cell culture medium was replaced with fresh medium containing fresh or freeze-dried condensed pDNA followed by incubation for 24 hours. The luciferase activity was measured using the luciferase chemiluminiscent assay kit (Promega) following the procedure described in the manual. The luminescence values were normalized with protein concentration. Condensed pDNA solutions were diluted 1:7 and the size distribution and ζ potential of pDNA complexes were measured with a ζ potential and particle size measurer (Malvern Instruments). pDNA and PEI were individually labeled with fluorescein (FITC) and rhodamine (RHO). The emission intensity from the solution at a wavelength of 520 nm was measured when the solution was excited at 488 nm. The degree of energy transfer was quantified by comparing the height of emission peak at 520 nm in the presence and absence of RHO-PEI.

Results:

Condensed pDNA was prepared by mixing pDNA with different types of PEI at various charge ratios of PEI to DNA. Fresh pDNA condensed with linear PEI led to higher gene expression than branched PEI. Freeze-drying condensed pDNA led to a reduction in gene expression, but the magnitude of decrease was greater with branched PEI (Fig. 1A). Linear PEI retained 43% of the gene expression of fresh condensed pDNA, while branched PEI retained only 10%. The size and ζ potential of the condensed pDNA were measured before and after freeze-drying. Freeze-drying brought about a significant decrease in the size while making a slight change in the ζ potential. The degree of energy transfer between fluorescently labeled PEI and pDNA was also measured before and after mixing heparin molecules with the fresh or freeze-dried PEI-pDNA complex. Recovery of donor (FITC) emission following the addition of heparin, corresponding to dissociation between PEI and pDNA, was much lower with freeze-dried PEI-pDNA complexes compared with fresh PEI-pDNA complexes (Fig. 1B).

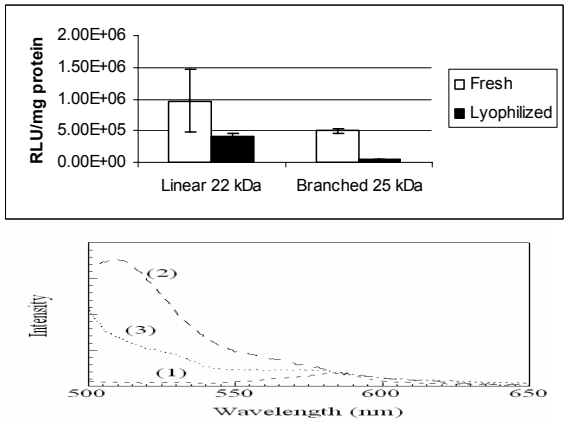

Fig. 1. Linear PEI led to a smaller decrease in gene expression caused by freeze-drying, compared with branched PEI (A). The freeze-drying process limits the recovery of donor (FITC) emission following the addition of heparin (B). Curve (1), (2), and (3) represent the emission curve from the PEI-pDNA complex, the mixture of fresh PEI-pDNA complex with heparin, and the mixture of freeze-dried PEI-pDNA complex with heparin, respectively.

Conclusions:

Our study demonstrates that the structure of PEI regulates transfection competence of pDNA-PEI condensates following freeze-drying. The freeze drying process greatly strengthened the association between PEI and pDNA, leading to a decrease in the size of PEI-pDNA complexes, and a reduction in gene expression. We propose that the pDNA condensates formed with linear PEI, while undergoing similar changes in structure following freeze drying, are capable of most efficient dissociation within the cellular cytoplasm and resultant increased gene expression, as compared with branched PEI.

References:

1. Pack DW, Hoffman AS, Pun S, Stayton PS. *Nat Rev Drug Discov.* 2005;4(7):581-93.
2. Anchordoquy TJ, Armstrong TK, Molina MC. *J Pharm Sci.* 2005;94(6):1226-36.