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Poly(ethylenimine) conjugated bioreducible dendrimer for efficient gene delivery

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ABSTRACT

Branched poly(ethylenimine) (PEI) 25 kDa is an efficient gene delivery vector with outstanding gene condensation ability and great endosome escape activity. However, it also induces higher cytotoxicity. Transfection efficiency and toxicity of PEI are highly dependent upon their molecular weight and structure. We developed a bioreducible poly(ethylenimine) (PEI (-s-s-)) derived from low molecular weight PEI (1.8 kDa) for efficient gene delivery. Bioreducible core molecule is expected to increase molecular weight and reduce the cytotoxicity of the copolymer. PEI (-s-s-) polyplexes showed higher transfection efficiency and lower cytotoxicity compared to branched PEI 25 kDa, Lipofectamine® 2000 and, FuGENE® 6. In addition, PEI (-s-s-) derivative (16 kDa) formed stable polyplexes with a zeta-potential value of +34 mV and polyplex size of 61 nm. PEI (-s-s-) derivative (16 kDa) showed excellent transfection efficiency: 3.6 times higher than branched PEI 25 kDa in HeLa cells and 7.4 times higher than Lipofectamine® 2000 in H9C2 cell. The derivatives also showed lower cytotoxicity compared with Lipofectamine® 2000 and PEI 25 kDa in various cell types. In addition, newly synthesized PEI (-s-s-) derivatives have high reproducibility.

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1. Introduction

Gene therapy has potential in treating many diseases, such as cancers, infectious diseases, and immune system disorders. Techniques for directly killing diseased cells, producing or inhibiting disease-related protein, and regulating the immune system have been applied to these diseases using gene therapy [1–4]. Efficient delivery of therapeutic genes to target cells is the most important step of gene therapy [2,5]. Both viral and non-viral delivery systems have been used for gene delivery [6]. Compared with viral vectors, non-viral vectors have advantages such as large-scale production, low immune responses, flexible loading capacity of therapeutic genes, and stability of the vector [7,8]. The development of efficient and safe non-viral delivery vectors is an important issue in non-viral gene therapy [9,10].

The typical non-viral vectors are cationic lipids and polymers. Among the cationic polymers, branched poly(ethylenimine) (PEI) 25 kDa is one of the most popular and inexpensive gene delivery vectors. Because of its high amine density, PEI shows outstanding gene encapsulation efficiency and great endosome escape activity. However, it could destabilize the cell membrane, which will cause cytotoxicity to the cell [11–14]. Transfection efficiency and toxicity of cationic polymer are highly dependent upon molecular weight and structure. For example, high molecular weight (HMW) PEI shows a high transfection efficiency but, it also induces higher cytotoxicity. Therefore, high toxicity of HMW PEI limits transfection to in vitro and in vivo conditions. Contrast, low molecular weight (LMW) PEI has lower cytotoxicity, but its transfection efficiency is very poor. Therefore, LMW PEI cannot be used as a non-viral gene delivery vector [15,16].

There have been several approaches to decrease the toxicity, while maintaining the merits of high transfection efficiency by combining PEI with biodegradable polymer to make an ideal non-viral vector. One way to overcome the cytotoxicity of cationic polymer is the introduction of biodegradable bonds such as ester and disulfide-bonds [17]. Nam et al. reported that the cytotoxicity of dendrimers could be decreased through the introduction of ester bond [18]. However, ester bonds are readily hydrolyzed in an aqueous environment. Contrast, disulfide bonds are not reduced until they are exposed to reducing agents such as ß-mercaptoethanol (BME), dithiothreitol (DTT), and glutathione (GSH) [19]. Therefore, disulfide-linked polymers are more stable than ester-linked polymers in the extracellular environment. In addition, disulfide bonds can be cleaved by glutathione in the intracellular cytoplasm [20]. Ou et al. synthesized and evaluated a family of bioreducible poly(disulfide amine)s as polymeric gene vectors. In that study, they showed that transfection efficiency and toxicity of cationic polymer are highly dependent upon molecular weight and structure [21]. Low molecular weight polymer required high weight ratio to indicate a high transfection efficiency. This was also associated with cytotoxicity. The disulfide-containing PEI derivatives were also reported by several groups. For example, Peng et al. reported that higher transfection efficiency could be obtained via thiolation of low molecular weight PEI (800 Da). In 2010, Koo et al. have synthesized biodegradable

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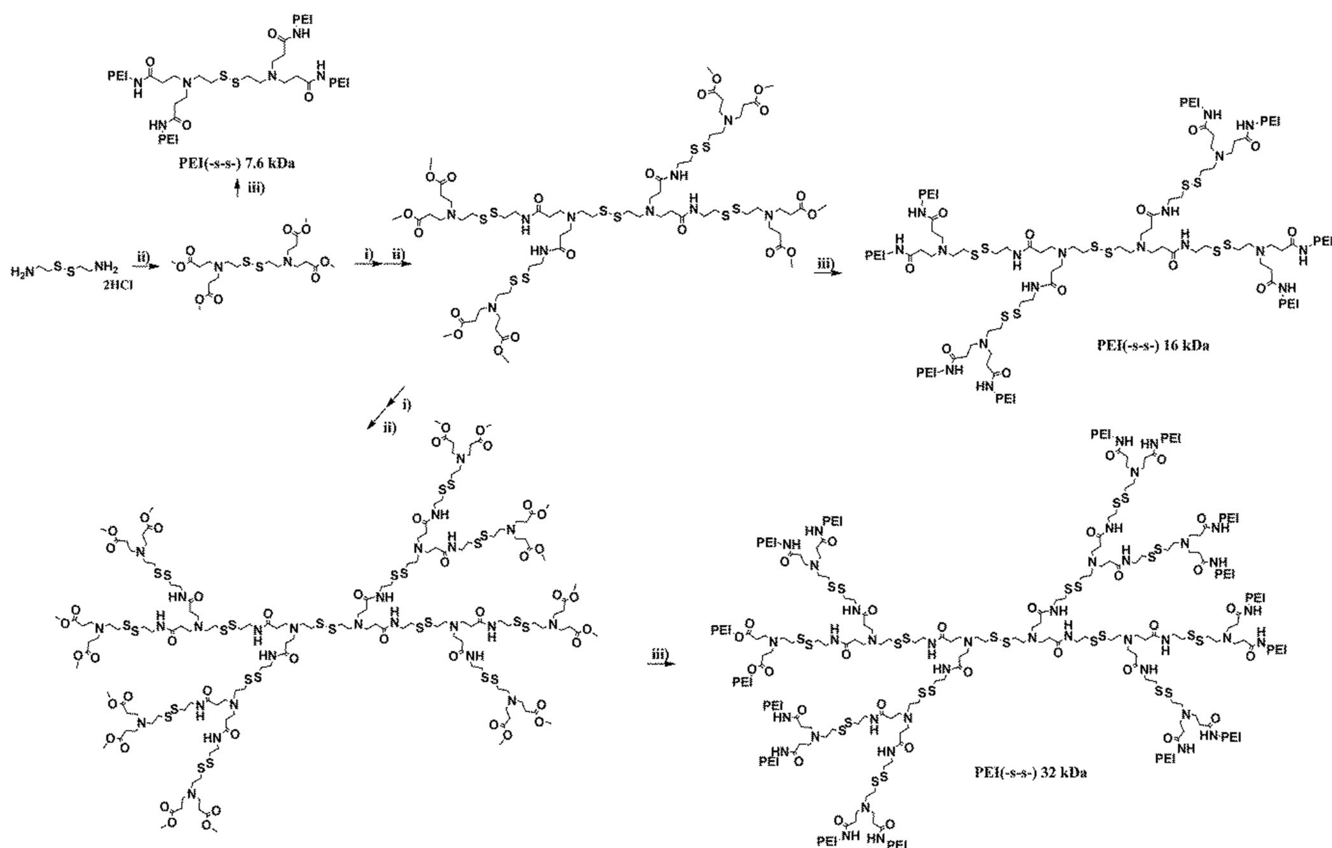

Fig. 1. The synthesis scheme and structure of bioreducible poly(ethylenimine) derivatives. i) Cystamine dihydrochloride, ii) methyl acrylate, iii) bPEI 1.8 kDa.

branched PEI by crosslinking linear PEIS which showed high transfection efficiency. Moreover, disulfide-containing PEIs were synthesized via click chemistry by Liu et al. [22–27].

Branched PEI consists 25, 50 and 25% of primary, secondary, and tertiary amine groups [28]. Because PEI has many primary amine groups, it can be easily modified to optimize its gene delivery activity and cytotoxicity [29]. To overcome the limitations of current PEI gene delivery systems, LMW PEI was connected with biodegradable core molecule. Bioreducible PEI (PEI (-s-s-)) was developed to impart biodegradable characteristics while maintaining the amine density.

Generally, the controlled synthesis of dendrimers results in low polydispersity while linear synthetic polymer has a high polydispersity. Moreover, dendrimer has well-defined numbers of terminal groups for the conjugation. In this study, we hypothesized that the conjugation of PEI 1.8 kDa with dendritic core molecule would increase the molecular weight of PEI, and that increasing molecular weight would also increase the transfection efficiency. In addition, we expected that polymers would have high stability because of using a disulfide bond rather than ester bond. We also expected that polymers would have a low polydispersity (P.D.) value because of the well-defined structure and surface functionality of dendritic core molecules. In this study, we described: 1) the synthesis and characterization of this novel bioreducible PEI derivatives; 2) its polyplex formation; 3) transfection efficiency; and 4) cytotoxicity.

Table 1
Molecular weights and second virial coefficients (A_2) of PEIs.

	Size exclusion chromatography		Static light scattering	
	kDa	Polydispersity	kDa	A_2 (mL mol/g^2)
PEI(-s-s-) 7.6 kDa	7.6	1.20	7.57 ± 0.41	−0.0336
PEI(-s-s-) 16 kDa	16.3	1.26	15.20 ± 0.82	−0.0168
PEI(-s-s-) 32 kDa	32.6	1.24	31.30 ± 1.70	−0.0081

2. Materials and methods

2.1. Materials

Branched polyethylenimine (bPEI 1.8 kDa, Mw 1800 Da) was purchased from Polysciences (Warrington, PA). Branched polyethylenimine (bPEI 25 kDa, Mw 25,000 Da), methanol (MeOH), cystamine dihydrochloride, triethylamine (TEA), methyl acrylate (MA), magnesium sulfate, diethyl ether, and thiazolyl blue tetrazolium bromide (MTT) were purchased from Sigma-Aldrich (St. Louis, MO). BCA protein assay kit was purchased from Pierce (Rockford, IL). The Luciferase assay system, reporter lysis buffer, and FuGENE® 6 were purchased from Promega (Madison, WI). Dulbecco's modified Eagle's medium (DMEM), Opti-MEM®, Dulbecco's phosphate buffered saline (DPBS), TrypLE™ Express, SYBR safe DNA gel stain, and Lipofectamine® 2000 were purchased from Invitrogen (Carlsbad, CA). Fetal bovine serum (FBS) was purchased from Seradigm (Radnor, PA). Dialysis membranes were purchased from Spectrum Laboratories (Rancho Dominguez, CA).

2.2. Preparation of bioreducible poly(ethylenimine)

2.2.1. Synthesis of 1st core molecule

Methyl acrylate (0.213 mol) was mixed with 10 mL of MeOH. Cystamine dihydrochloride (8.881 mmol) and TEA (0.018 mol) were dissolved in 20 mL of MeOH and cystamine solution was added dropwise to methyl acrylate solution over 30 min. After two days of reaction at room temperature under a nitrogen atmosphere, MeOH and TEA were removed by evaporation. A viscous light yellow liquid interspersed with white precipitate was dissolved in diethyl ether and extracted

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