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Arginine-conjugated polypropylenimine dendrimer as a non-toxic and efficient gene delivery carrier

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Abstract

We synthesized arginine-conjugated polypropylenimine dendrimer G2 (DAB-8), PPI2-R for gene delivery systems. Synthesized PPI2-R could retard plasmid DNA at a weight ratio of 4 completely and PPI2-R polyplexes showed a fluorescence of less than 10% over a charge ratio of 2 by PicoGreen reagent assay, suggesting its good DNA condensing ability. The size of PPI2-R polyplex was measured to about 200 nm at a charge ratio of 150. PPI2-R displayed 80–90% cell viability at even a 150 μg/mL concentration. Transfection efficiency of PPI2-R was found to be high comparable to that of PEI25kD and to be 8–214 times higher than that of unmodified PPI2 on HeLa and 293 cells. Moreover, PPI2-R showed 4 times higher transfection efficiency than PEI25kD, treating with 10 μg pDNA because of its low cytotoxicity on HeLa cells. Finally, PPI2-R showed a transfection efficiency 2–3 times higher than PEI25kD on HUVECs, showing its potency as a gene delivery carrier for primary cells. These results demonstrate that arginine-conjugation of PPI2 is successful in developing a low toxic and highly transfection efficient gene delivery carrier.

Keywords: Gene delivery; Polypropylenimine dendrimer; Arginine-conjugation; Low cytotoxicity; High transfection

1. Introduction

A number of non-viral gene delivery carriers including cationic polymers and lipids have been developed as alternatives to viral gene delivery carriers due to their advantages such as non-immunogenicity, unlimited capacity of genes delivered, and convenient handling and easy introduction of functionalities in spite of relatively low transfection efficiency [1–4].

Among them, dendrimers aroused researchers' interest for drug and gene delivery systems because they have unique and interesting characteristics such as defined structures, inner cavities able to encapsulate guest molecules, and controllable multi-valent functionalities in their inner or outer part [5–9].

However, difficulty of synthesizing dendrimers and fitness of dendrimers as drug delivery carriers have led mainly to modification of existing dendrimers, not development of novel dendrimers for gene delivery systems. Among them, poly(amido amine (PAMAM) dendrimers and polypropylenimine (PPI) dendrimers are representative existing dendrimers.

PAMAM dendrimers have been utilized and examined for gene delivery systems in vitro and in vivo, extensively [10,11]. PAMAM dendrimers have been also modified with PEG, amino acids, or ligands in order to enhance the gene delivery potency [12–16].

In contrast, applications of PPI dendrimers for gene delivery have been limited to a small number of works. The gene delivery results of PPI dendrimers and quaternized PPI dendrimers were mainly reported by Uchegbu and colleagues [17,18]. Also, self-assembled ternary complexes of PPI dendrimer, cucurbituril, and DNA were examined as a novel non-covalent strategy for gene delivery systems by Lim et al. [19].

PAMAM dendrimer conjugated with arginine residues was previously reported to show enhanced transfection efficiency [13]. Also, replacement of terminal lysine with arginine was reported to enhance the transfection efficiency

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in dendritic poly(L-lysine) analogs by Okuda et al. [20]. This may be due to the membrane permeability and nuclear localization ability of arginine residues conjugated to the periphery of the dendrimers. So, we tried to conjugate arginine residues to PPI dendrimer in order to study the arginine conjugation effect of PPI dendrimer for the first time.

Here, we report the synthesis of arginine-conjugated PPI dendrimer, PPI2-R and its characterization including cytotoxicity and transfection efficiency for gene delivery systems.

2. Materials and methods

2.1. Materials

Poly(ethylenimine) (25 kDa), PPI octaamine dendrimer G2.0 (DAB-Am-8), *N*,*N*-diisopropylethylamine (DIPEA), piperidine, and 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) were purchased from Sigma–Aldrich (St. Louis, MO). *N*-hydroxybenzotriazole (HOBt), 2-(1H-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU) were purchased from Anaspec, Inc. (San Jose, CA). Fmoc-L-Arg(pbf)-OH was purchased from Novabiochem (San Diego, CA). Luciferase 1000 Assay System and Reporter Lysis Buffer were purchased from Promega (Madison, WI). The luciferase expression plasmid, pCN-Luci was constructed by subcloning cDNA of Photinus pyralis luciferase with 21-amino acid nuclear localization signal from SV40 large T antigen to pCN [21]. PicoGreen reagent was purchased from Molecular Probes (Eugene, OR). Fetal bovine serum (FBS), and Dulbecco's modified Eagle's medium (DMEM) were purchased from GIBCO (Gaithersburg, MD). EGM and EGM-2 MV SingleQuots medium were purchased from Cambrex Bio Science (Walkersville, MD). All chemicals were used without any further purification.

2.2. Synthesis of PPI2-R

PPI2-R was synthesized by conjugating arginine residues to the periphery of PPI2 dendrimer as shown in Fig. 1. Firstly, PPI2 dendrimer was weighed and dissolved in anhydrous DMF. Arginine conjugation reaction was performed in anhydrous DMF for 2 days at room temperature with 32 equivalents of HOBt, HBTU, Fmoc-Arg(pbf)-OH, and 64 equivalents of DIPEA. After completion of the reaction, the reaction mixture was precipitated twice with an excess of diethyl ether. Residual precipitate was dissolved in DMF and mixed with an equal volume of piperidine (30% in DMF, v/v) at room temperature for 20 min to remove the Fmoc groups of coupled Fmoc-Arg(pbf)-OH and precipitated again with diethyl ether. Then, the reagent (95:2.5:2.5, trifluoroacetic acid/triisopropylsilane/water, v/v) was used to deprotect the pbf groups of coupled arginine residues at room temperature for 1 h. After the reaction, the final product, PPI2-R was dialyzed by dialysis membrane (Spectrum Laboratories, Inc., Rancho Dominguez, CA, MWCO = 1000) against ultra-pure water for 4 h and lyophilized before use for analysis and assay.

2.3. ^1H NMR spectroscopy

^{1}H NMR spectra of the polymers were obtained using a Bruker DPX-300 NMR spectrometer (300 MHz). For analysis, the polymer samples were dissolved in D_2O containing 3-(trimethylsilyl)propionic-2,2,3,3-*d*4 acid sodium salt as an internal reference (0 ppm).

2.4. Gel retardation assays

PPI2-R/plasmid complexes at various weight ratios ranging from 1 to 10, were prepared in Hepes buffered saline (10 mM Hepes, 1 mM NaCl, pH 7.4). After 30 min incubation at room temperature for the complex formation, the samples were electrophoresed on a 0.7% (w/v) agarose gel and stained in an ethidium bromide solution (0.5 μg/mL), and analyzed on a UV illuminator to show the location of the DNA.

2.5. Polymer/DNA self-assembly analysis by PicoGreen assay

PicoGreen reagent (200 ×) was diluted 200-fold in TE buffer before the experiment. About 200 μL of the diluted PicoGreen stock solution was mixed with the same volume of blank solution or polyplex solution (1 μg DNA, 1 × HBS) prepared at various charge ratios ranging from 1 to 10. After 2 min incubation, each solution was added to 1.6 mL of TE buffer in a test tube. Fluorescence was measured with a FP-750 spectrofluorometer (Jasco) at room temperature. Excitation and emission wavelengths were set at 480 and 520 nm, respectively. Results were represented as relative fluorescence (%) to DNA control.

2.6. Polyplex size measurements

The hydrodynamic diameters of the PPI2-R/plasmid DNA complexes were determined by light scattering. About 2 mL of polyplex solutions containing 5 μg of DNA were prepared at various charge ratios ranging from 10 to 200. After 30 min incubation, polyplex sizes were measured using a Zetasizer 3000HS (Malvern Instruments, UK). The laser used was a nominal 5 mW HeNe laser having a 633 nm wavelength. Scattered light was detected at a 90° angle. The refractive index (1.33) and the viscosity (0.89) of ultrapure water were used at 25 °C for measurements. Zetasizer 3000 (Advanced) Size mode v1.61 software was used for data acquisition. Data analysis was performed in automatic mode. Measured sizes were represented as the average values of 5 runs.

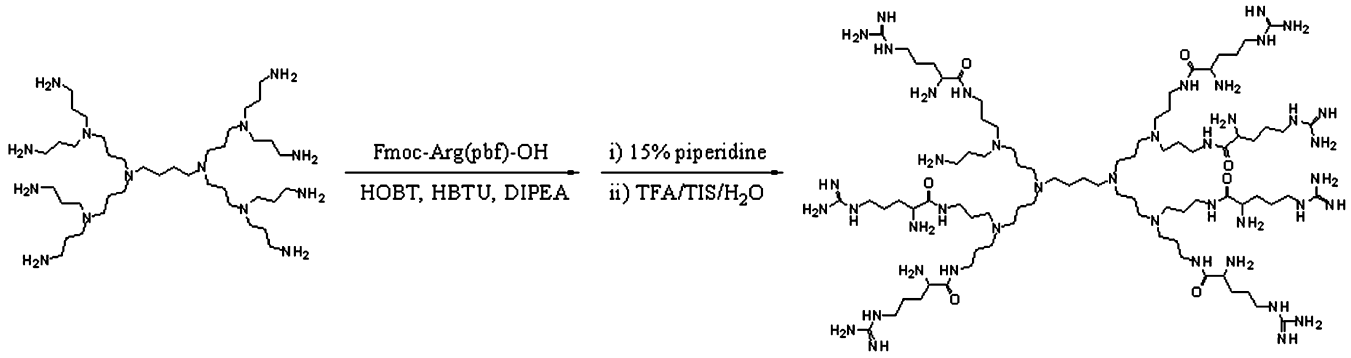

Fig. 1. The synthetic scheme of PPI2-R.