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Cationic star polymers consisting of α-cyclodextrin core and oligoethylenimine arms as nonviral gene delivery vectors

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Abstract

A series of novel cationic star polymers were synthesized by conjugating multiple oligoethylenimine (OEI) arms onto an α-cyclodextrin (α-CD) core as nonviral gene delivery vectors. The molecular structures of the α-CD-OEI star polymers, which contained linear or branched OEI arms with different chain lengths ranging from 1 to 14 ethylenimine units, were characterized by using size exclusion chromatography, ^{13}C and ^{1}H NMR, and elemental analysis. The α-CD-OEI star polymers were studied in terms of their DNA binding capability, formation of nanoparticles with plasmid DNA (pDNA), cytotoxicity, and gene transfection in cultured cells. All the α-CD-OEI star polymers could inhibit the migration of pDNA on agarose gel through formation of complexes with pDNA, and the complexes formed nanoparticles with sizes ranging from 100 to 200 nm at N/P ratios of 8 or higher. The star polymers displayed much lower *in vitro* cytotoxicity than that of branched polyethylenimine (PEI) of molecular weight 25K. The α-CD-OEI star polymers showed excellent gene transfection efficiency in HEK293 and Cos7 cells. Generally, the transfection efficiency increased with an increase in the OEI arm length. The star polymers with longer and branched OEI arms showed higher transfection efficiency. The best one of the star polymers for gene delivery showed excellent *in vitro* transfection efficiency that was comparable to or even higher than that of branched PEI (25K). The novel α-CD-OEI star polymers with OEI arms of different chain lengths and chain architectures can be promising new nonviral gene delivery vectors with low cytotoxicity and high gene transfection efficiency for future gene therapy applications.

Keywords: Cyclodextrin; Star polymer; Polyethylenimine; Gene transfer

1. Introduction

Cationic polymers are the major type of the nonviral gene delivery vectors investigated in the past decade [1–6]. A great number of polycations have been reported to be able to effect gene transfection, including homopolymers or copolymers of polyethylenimine (PEI) [7], poly(L-lysine) [4], polyamidoamine [8], poly(L-glutamic acid) [9], polyphosphoester [10,11], and chitosan [12,13]. Among these polymers, PEI homopolymers with a molecular weight (MW) higher than 25K are currently the most popular polymers used as gene carriers. They are considered the gold standard for polymeric nonviral gene delivery due to their high transfection efficiency, but the rather high toxicity of these PEI homopolymers strictly limits their application in gene therapy. Meanwhile, it is generally believed that PEI homopolymers with a MW less than 1.8K shows low gene delivery ability but is less toxic [14].

Cyclodextrins (CDs) are a series of cyclic oligosaccharides composed of 6, 7, or 8 D(+)-glucose units linked by α-1,4-linkages and named α-, β-, or γ-CD, respectively. They are biocompatible, and do not elicit immune responses and have low toxicities in animal and human bodies [15]. Since 1999, a class of linear and CD-based polymers were introduced by Davis and co-workers for the delivery of nucleic acids [16–20]. Most of these polymers contained amines and CDs in the polymer backbone. Uekama's group also conjugated CDs (α-, β- or γ-CD) to

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polyamidoamine dendrimers to enhance gene transfection [21,22]. Further, Davis' and Pack's groups modified PEI with β-CD, grafting multiple β-CD molecules to linear or branched PEI (MW 25K) [20,23]. These CD-grafted PEIs delivered nucleic acids efficiently to cultured cells with lower toxicity than the pristine PEI (MW 25K). Recently, Tang et al. also used CDs to crosslink low MW branched PEI (MW 600) to form high MW cationic polymers (average MW 61K), which displayed lower cytotoxicity and high gene transfection in cultured cells [24]. Most recently, we reported the synthesis of novel cationic supramolecules composed of multiple oligoethylenimine-grafted β-CD that are threaded and blocked on a polymer chain as a new class of polymeric gene delivery vectors [25]. In contrast to the conventional cationic polymers containing a long sequence of covalently bonded repeating units, the novel supramolecular gene carriers were designed based on a new mechanism, where many cationic cyclic units were threaded upon a polymer chain to form an integrated supramolecular entity to function as a macromolecular gene vector, which showed excellent DNA binding ability, low cytotoxicity, and high gene transfection efficiency.

In this study, we synthesized a series of new cationic star polymers where many oligoethylenimine (OEI) arms of different lengths are attached to an α-CD core. We found that not only these new α-CD-OEI star polymers showed much lower cytotoxicity, but the gene transfection efficiency is similar to that of branched PEI (MW 25K). We also investigated the effect of the length of the OEI arms of the star polymers, and found that the transfection efficiency of the α-CD-OEI star polymers improved with increase of the chain length of the OEI arms.

2. Experimental methods

2.1. Materials

Pentaethylenehexamine was obtained from Fluka. α-CD was purchased from Tokyo Kasei incorporation. Ethylenediamine, linear PEI with MW of 423 (OEI-9), branched PEI with MW of 600 (OEI-14) and branched PEI (MW 25K) were supplied by Aldrich. D_2O used as solvent in the NMR measurements was also obtained from Aldrich. Qiagen kit and Luciferase kit were purchase from Qiagen and Promega, respectively. 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT), penicillin, and streptomycin were obtained from Sigma.

2.2. Synthesis of α-CD-OEI star polymers 1–4

Scheme 1 shows the synthesis procedures and the structures of alpha –CD-OEI star polymers. The details of the preparation of star polymer **2** are given below as a typical example. α-CD (0.414 g, 0.4 mmol, 94% purity) was dried at 120 °C in vacuum overnight. When the flask cooled, 40 mL dry DMSO was injected under nitrogen. After all α-CD was dissolved, the DMSO solution of α-CD was added dropwise during a period of 6 h under nitrogen to 40 mL of anhydrous DMSO solution in which 1,1′-carbonyldiimidazole (CDI) (5.84 g, 36 mmol) was dissolved, and the mixture was stirred overnight under nitrogen at room temperature. Then, the mixture of 400 mL THF and 1700 mL Et_2O was poured in the resulting solution to precipitate the product. The precipitate was centrifuged and washed with THF three times. Then, the resulting sticky solid was dissolved in 40 mL DMSO and this solution was slowly added dropwise during a period of 3 h into 12.55 mL (43.2 mmol) of pentaethylenehexamine which was dissolved in 40 mL of DMSO with stirring at room temperature, followed by stirring the mixture overnight; 900 mL THF was poured in the reaction mixture to precipitate the product. The precipitate was centrifuged and washed with THF three times, and the resulting crude product was purified by size exclusion chromatography (SEC) on a Sephadex G-50 column using DI water as eluent. Finally, 0.189 g white solid **2** was obtained.

In the case of preparation of star polymer **4**, dichloromethane (DCM) was used to precipitate the product instead of THF. Then, the reaction mixture was filtered and the solid was washed with DCM three times. Finally, the resulting crude product was purified by SEC on a Sephadex G-50 column using DI water as eluent.

The yields and analytical data for all four products are given below.

α-CD-OEI star polymer **1**. Yield, 21%. ^{1}H NMR (400 MHz, D_2O, 22 °C): δ5.14 (s, broad, 6H, H(1) of CD), 3.47–4.62 (m, broad, 36H, H(3), H(6), H(5), H(2) and H(4) of CD), 2.98–3.47 (t, broad, 12H, methylene of $-CONHCH_2-$), 2.86 (s, broad, 12H, methylene of $-CH_2NH_2$). Anal. Calcd. for $C_{53}H_{95}N_{12}O_{36}\cdot 6H_2O$: C, 40.59; H, 6.81; N, 10.27. Found: C, 40.35; H, 6.30; N, 10.07.

α-CD-OEI star polymer **2**. Yield, 17%. ^{1}H NMR (400 MHz, D_2O, 22 °C): δ4.98 (d, broad, 6H, H(1)H of CD), 3.40–4.64 (m, broad, 36H, H(3), H(6), H(5), H(2) and H(4) of CD), 2.92–3.40 (t, broad, 14H, methylene of $-CONHCH_2-$), 2.65 (m, 123H, ethylene of pentaethylenehexamine). Anal. Calcd. for $C_{111}H_{237}N_{41}O_{37}\cdot 4H_2O$: C, 47.50; H, 8.81; N, 20.41. Found: C, 47.66; H, 8.96; N, 20.71.

α-CD-OEI star polymer **3**. Yield, 29%. ^{1}H NMR (400 MHz, D_2O, 22 °C): δ5.12 (d, broad, 6H, H(1) of CD), 3.38–4.65 (m, broad, 36H, H(3), H(6), H(5), H(2) and H(4) of CD), 2.90–3.38 (m, broad, 7H, methylene of $-CONHCH_2-$), 2.67 (m, 122H, ethylene of OEI-9). Anal. Calcd. for $C_{104}H_{226}N_{39}O_{33}\cdot 5H_2O$: C, 48.05; H, 9.11; N, 21.13. Found: C, 47.63; H, 8.53; N, 21.07.

α-CD-OEI star polymer **4**. Yield, 19%. ^{1}H NMR (400 MHz, D_2O, 22 °C): δ5.11 (s, broad, 6H, H(1) of CD), 3.46–4.60 (m, broad, 36H, H(3), H(6), H(5), H(2) and H(4) of CD), 3.00–3.46 (m, broad, 10H, methylene of $-CONHCH_2-$), 2.67 (m, 264H, ethylene of OEI-14). Anal. Calcd. for $C_{179}H_{409}N_{74}O_{35}\cdot 24H_2O$: C, 43.97; H, 9.53; N, 19.44. Found: C, 42.63; H, 8.65; N, 20.79.

2.3. Analytical methods

Gel permeation chromatography (GPC) analysis was carried out with a Shimadzu SCL-10A and LC-10AT system equipped with a Sephadex G-75 column (size: 2.5 × 32 cm), a Shimadzu RID-10A refractive index detector. PBS buffer solution (1 ×) was used as the eluent. Fractions were collected per 1 mL and were further detected with a HORIBA SEPA-300 high speed accurate polarimeter at wavelength 589 nm with cell length 10 cm and response 2 s.

The ^{1}H NMR spectra were recorded on a Bruker AV-400 NMR spectrometer at 400 MHz at room temperature. The ^{1}H NMR measurements were carried out with an acquisition time of 3.2 s, a pulse repetition time of 2.0 s, a 30° pulse width, 5208-Hz spectral width, and 32K data points. Chemical shifts were referred to the solvent peaks ($\delta = 4.70$ ppm for D_2O).

The ^{13}C NMR spectra were recorded on a Bruker AV-400 NMR spectrometer at 100 MHz at room temperature. The ^{13}C NMR measurements were carried out using composite pulse decoupling with an acquisition time of 0.82 s, a pulse repetition time of 5.0 s, a 30° pulse width, 20,080-Hz spectral width, and 32K data points.

2.4. Plasmid

The plasmid used was pRL-CMV (Promega, USA), encoding *Renilla* luciferase, which was originally cloned from the marine organism *Renilla reniformis*. The plasmid DNA was amplified in *E. coli* and purified according to the supplier's protocol (Qiagen, Hilden, Germany). The