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Water soluble cationic dextran derivatives containing poly(amidoamine) dendrons for efficient gene delivery

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ABSTRACT

To develop new dextran derivatives for efficient gene delivery, the conjugation of poly(amidoamine) dendrons onto biocompatible dextran was carried out by a Cu(I)-catalyzed azide-alkyne cycloaddition, as confirmed by FTIR and ^{1}H NMR analyses. For resultant dextran conjugates with various generations of poly(amidoamine) dendrons, their buffering capacity and in vitro cytotoxicity were evaluated by acid–base titration and MTT tests, respectively. In particular, their physicochemical characteristics for the complexation with plasmid DNA were investigated by the combined analyses from agarose gel electrophoresis, zeta potential, particle size, transmission electron microscopy and fluorescence emission spectra. Moreover, their complexes with plasmid DNA were studied with respect to their transfection efficiency in human embryonic kidney (HEK293) cell lines. In the case of a higher generation of poly(amidoamine) dendrons, such a dextran conjugate was found to have much lower cytotoxicity and better gene delivery capability when compared to branched polyethylenimine (bPEI, 25 kDa), a commonly used gene vector.

1. Introduction

Recent advances in gene delivery technology have focused on developing more biocompatible and non-cytotoxic nonviral vectors, in particular water soluble cationic polysaccharide derivatives (Buschmann et al., 2013; Hu, Zhao, Yu, Liu, & Xu, 2014; Khan et al., 2012; Raemdonck, Martens, Braeckmans, Demeester, & De Smedt, 2013; Song et al., 2015; Tiera, Shi, Winnik, & Fernandes, 2011; Xiu, Zhao, Yang, & Xu, 2013). Among various polysaccharides used for the development of gene vectors, dextran consisting of α-1,6 glycosidic straight linkages with a few β-1,3 glycosidic branch linkages is an attractive candidate because of its biodegradability, biocompatibility, and non-immunogenicity (Hosseinkhani, Aoyama, Ogawa, & Tabata, 2003). Up to now, some cationic dextran derivatives have been synthesized and investigated for gene delivery. For example, Li et al. (2013) prepared dextran-graft-poly((2-dimethyl amino)ethyl methacrylate) as an efficient delivery system for tumor gene therapy; Thomas, Rekha, and Sharma (2010) prepared cationic dextran–protamine conjugate as a haemocompatible and nonviral gene vector; Yeo et al. (2014) conjugated spermine onto dextran for delivering gene to the lung of mouse via intranasal route. However, the efficiency of gene delivery by these cationic dextran derivatives is still relatively low when compared to commonly used polyethylenimine (PEI) or cationic lipids. Therefore, there has been growing interest in the development of new dextran-based gene carriers with high transfection efficiency.

In this work, we carried out for the first time the chemical modification of dextran by the click conjugation of poly(amidoamine) (PAMAM) dendrons for the delivery of plasmid pEGFP-N3 to human embryonic kidney (HEK293) cells. For resultant dextran conjugates with various generations of poly(amidoamine) dendrons, their buffering capacity, cytotoxicity, and complexation with plasmid pEGFP-N3 as well as the gene transfection in the absence and presence of serum were studied. Moreover, their feasibility as a non-viral gene vector was investigated further by the comparative studies with branched polyethylenimine, one of the most potent synthetic gene delivery vectors (Jäger., Schubert, Ochrimenko, Fischer, & Schubert, 2012).

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2. Materials and methods

2.1. Materials

Dextran (M_w = 40 kDa) was purchased from Sinopharm Chemical Reagent Shanghai Co. Ltd. (China) and used as received. By GPC measurement (Fig. S1), its number-average molecular weight (M_n) and molecular weight distribution (M_w/M_n) were determined to be 33,489 and 1.55, respectively. By ^{1}H NMR analysis (Benzeval, Bowyer, & Hubble, 2012; Pasika & Cragg, 1963; Vettori, Franchetti, & Contieroa, 2012), its degree of α-(1,3)-linked branching was found to be 5.0% (Fig. S2). Branched polyethylenimine (bPEI, M_w = 25,000) and propargylamine (98%) were purchased from Sigma–Aldrich Co. LLC (USA). Sodium tetrahydridoborate ($NaBH_4$, A.R.) and ethylenediamine (EDA, A.R.) were purchased from Aladdin Reagent Shanghai Co. Ltd. (China). Sodium ascorbate (99%) and ethidium bromide (EtBr, 98%) was purchased from Alfa Aesar Co. Ltd. Methyl acrylate (A.R.) was obtained from Guangzhou Chemical Reagent company (China) and dried with anhydrous calcium chloride following by distillation under reduced pressure before used. 3-Azidopropargylamine (3-APA) was prepared according to the method reported previously (Carboni, Benalil, & Vaultier, 1993; Jiang, Zhang, Zhou, Xu, & Liu, 2008). The plasmid pEGFP-N3 (pDNA, 4.7 kb), which encodes for green fluorescent protein (GFP), was purchased from Clontech Laboratories Inc. (Japan) and used as a gene reporter in all experiments. 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) was purchased from Invitrogen Corporation (Washington, USA). Deoxyribonuclease I (DNase I) was purchased from Fermentas Life Sciences. The Dulbecco's modified Eagle medium (DMEM), trypsin–ethylenediaminetetraaceticacid (Trypsin–EDTA), and fetal bovine serum (FBS) were purchased from Gibco-BRL (Canada). All other reagents were of analytical grade and used without further purification.

2.2. Preparation of propargyl focal point PAMAM dendrons

The propargyl focal point PAMAM dendrons with three or four generations (Dm, m = 3, 4) were synthesized according to a protocol similar to that described by Lee et al. (2006), as shown in Scheme S1 in supplementary. Briefly, a solution of propargylamine (2.000 g, 36.3 mmol) in methanol (5 mL) was added dropwise to the ice-water bath cooling solution of methyl acrylate (7.830 g, 90.9 mmol) in methanol (10 mL). The reaction mixture was stirred vigorously for 1 h in the ice-water bath and 48 h at room temperature. The resultant solution was evaporated under reduced pressure, and then the residue was dried in vacuo at 35 °C to give the methyl ester-terminated dendron of D0.5 (7.980 g, 97%). The solution of D0.5 (6.070 g, 26.7 mmol) in methanol (20 mL) was added dropwise to the ice-water bath cooling solution of EDA (32.100 g, 0.534 mol) in methanol (30 mL) over a period of 1 h. The reaction mixture was stirred vigorously for 48 h at room temperature, and then evaporated under reduced pressure. The excess EDA was removed by azeotropic distillation (toluene/methanol = 9:1, v/v). The residue was dried in vacuo at 35 °C to obtain the amino-terminated PAMAM dendron of D1 as a yellow oil (7.260 g, 96%). The amino-terminated PAMAM dendrons (D3 and D4) were synthesized using the similar method above by successive Michael addition of primary amines with methyl acrylate and amidation of methyl ester groups with a large molar excess of EDA.

Propargyl focal point PAMAM dendron with three generations (PAMAM D3): ^{1}H NMR (300 MHz, $CDCl_3$): δ (ppm) = 1.66 (16H, —NH_2), 2.24 (1H, HC≡C—), 2.35 (28H, —CH_2CONH—), 2.52 (12H, —$NHCH_2CH_2N(R)$—), 2.75 (16H, —CH_2NH_2), 2.81 (28H, —$N(R)CH_2CH_2CO$—), 3.31 (28H, —$CONHCH_2$—), 3.48 (2H, HC≡CCH_2—), 7.21–7.97 (14H, —CONH—).

Propargyl focal point PAMAM dendron with four generations (PAMAM D4): ^{1}H NMR (300 MHz, $CDCl_3$): δ (ppm) = 1.74 (32H, —NH_2), 2.20 (1H, HC≡C—), 2.37 (60H, —CH_2CONH—), 2.58 (28H, —$NHCH_2CH_2N(R)$—), 2.71 (32H, —CH_2NH_2), 2.83 (60H, —$N(R)CH_2CH_2CO$—), 3.26 (60H, —$CONHCH_2$—), 3.45 (2H, HC≡CCH_2—), 7.03–7.87 (30H, —CONH—).

2.3. Preparation of azidized dextran

Azidized dextran (Dex-N_3) was prepared by a similar modification procedure reported by Azzam et al. (2002), as shown in Scheme S2 in supplementary. Firstly, the oxidized dextran was synthesized by periodate oxidation of dextran. In brief, a solution of dextran (5.000 g, 30.9 mmol of glucose units) dissolved in deionized water (70 mL) was stirred vigorously in the ice-water bath, to which the solution of sodium iodate (1.650 g, 7.72 mmol) dissolved in deionized water (10 mL) was slowly added. The resulting mixture was stirred in dark at room temperature for 12 h until a clear yellow solution was obtained, and then ethylene glycol (2 mL) was added under stirring for another 4 h until the color of the solution did not change anymore. The resultant solution was dialyzed (MWCO = 8–14 kDa) against deionized water for 2 days, and then lyophilized to obtain oxidized dextran (4.420 g) as a white solid. The aldehyde group content of oxidized dextran was determined to be 37.9% by the hydroxylamine hydrochloride method (Zhao & Heindel, 1991). Secondly, 3-APA (0.740 g, 7.40 mmol) was added to the solution of oxidized dextran (2.100 g, 4.92 mmol of —CHO groups) dissolved in borate buffer (40 mL, pH ~ 8.5, 0.1 mol/L of borax and 0.4 mol/L of NaCl). The reaction mixture was stirred in dark for 2 days. After adding $NaBH_4$ (0.280 g, 7.32 mmol) in the ice-water bath, the stirring was allowed to be continued for another 2 days to yield a light-yellow mixture. The resultant mixture was dialyzed (MWCO = 8–14 kDa) against deionized water for 2 days, and then lyophilized to produce the azidized dextran (Dex-N_3, 1.110 g) as a light brown solid. The azido group content of Dex-N_3 was determined to be 12.2% by elemental analysis (EA).

Dex-N_3: FT-IR (KBr, cm^{-1}): 3366 (ν_{O-H}), 2918 (ν_{C-H}), 2104 (—N_3), 1631 (δ_{O-H}), 1458 (δ_{H-C-H}), 1354 (δ_{C-H}), 1106 (δ_{C-O-C}), 1149 (δ_{C-C}), 1011 (δ_{C-O-H}). ^{1}H NMR (300 MHz, D_2O): δ (ppm) = 4.84 (proton connects to anomeric carbon), 3.99 (—CH_2N_3), 3.93–3.30 (protons on anhydroglucose unit), 3.08 (—$NHCH_2CH_2$—), 1.86 (—$CH_2CH_2N_3$). EA: 40.47% C, 6.780% H, 3.972% N (theoretical 44.22% C, 6.651% H, 3.955% N).

2.4. Click reaction between propargyl focal point PAMAM dendrons and azidized dextran

The click reaction between propargyl focal point PAMAM dendron (D3 or D4) and azidized dextran (Dex-N_3) was carried out by a Cu(I)-catalyzed azide-alkyne cycloaddition, as shown in Scheme S3 in supplementary. Briefly, Dex-N_3 (0.075 g, 0.05 mmol of azido groups) and propargyl focal point PAMAM dendron (0.16 mmol, 0.260 g for D3, 0.560 g for D4, respectively) were dissolved in dimethyl sulfoxide (8 mL), following by successive addition of $CuSO_4 \bullet 5H_2O$ (0.013 g, 0.05 mmol) and sodium ascorbate (0.020 g, 0.11 mmol) in aqueous solution. The reaction mixture was stirred at 35 °C for 24 h, and then dialyzed against deionized water for 2 days with the dialysis tubing (MWCO = 8–14 kDa). After the lyophilization of the clear yellow solution, the conjugate of Dex-N_3 with PAMAM D3 or PAMAM D4 was obtained as a yellow solid (0.160 g in the case of PAMAM D3, and 0.251 g in the case of PAMAM D4). The PAMAM dendron content was determined by elemental analysis to be 11.8% for the conjugate (Dex-D3) of Dex-N_3 with PAMAM D3 and 11.7% for the conjugate (Dex-D4) of Dex-N_3 with PAMAM D4, respectively.