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Multi-armed poly(L-glutamic acid)-graft-polypropyleneinime as effective and serum resistant gene delivery vectors

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ABSTRACT

A new series of multi-armed MP-g-PPI dendrimers were synthesized by polymerization of BLG-NCA using G2.0PPI as macromolecular initiator and subsequent aminolysis with G1.0PPI or G2.0PPI. The chemical structure and composition of the MP-g-PPI dendrimers were characterized by Fourier transform infrared spectroscopy (FT-IR) and nuclear magnetic resonance spectroscopy (^{1}H NMR). The MP-g-PPI showed a great ability to combine with pDNA to form complexes, which protect the pDNA from nuclease degradation. Dynamic light scattering (DLS) measurement illustrated that the sizes of complexes were in range of 111–219 nm. The transmission electron microscope (TEM) and atomic force microscope (AFM) observation showed that the morphology of these complexes was spherical. The MTT assay demonstrated that cytotoxicity of the MP-g-PPI was lower than that of PEI 25 K. The in vitro transfection test indicated that MP-g-PPI gene vectors displayed relative high transfection efficiency than that of PEI 25 K and Lipofectamine 2000 in serum-containing medium. Furthermore, MP-g-PPI at the weight ratio of 7.5 displayed better serum-resistant capability than that of PEI 25 K and Lipofectamine 2000. The above facts revealed that multi-armed MP-g-PPI dendrimers may be promising gene vectors with low cytotoxicity, high transfection efficiency and serum-resistant ability.

1. Introduction

Gene therapy is a promising new method for the treatment of inherited and acquired disease (Conley and Naash, 2010). Compared to viral gene delivery vectors, synthetic cationic polymers have been increasingly investigated as safe alternatives for gene therapy (Sun,et al., 2008; Park et al., 2008) because of their potential advances such as the large DNA loading capacity, the absence of immune response, and the flexibility to design a vector with well-defined structural and chemical properties (Lee et al., 2008; Rahbek et al., 2008; Chen et al., 2009).

Dendrimers are synthetic macromolecules with well-defined, highly branched molecular structure that are synthesized in an algorithmic step-by-step fashion, such as polyamidiamine (PAMAM) dendrimer and polypropyleneimine (PPI) dendrimer (Tack et al., 2006a,b). Among dendrimers, PAMAM dendrimers have been utilized and examined for gene delivery vectors in vitro and in vivo, extensively (Liu et al., 2013; Ziraksaz et al., 2013; Wang et al., 2011). PAMAM dendrimers have been also modified with PEG, amino acids, or ligands in order to enhance the gene delivery capacity (Yin et al., 2012; Wen et al., 2012; Arima et al., 2012; Bai et al., 2013). On the contrary, the applications of PPI dendrimers for gene delivery have been limited to a small number of works due to their cytotoxicity in high generation polypropylenimine dendrimers (Tack et al., 2006a,b). In recent years, low generation polypropylenimine dendrimers have been gradually notified because of their extremely low cytotoxicity and studied as gene delivery systems (Zhang et al., 2008; Zinselmeyer et al., 2002). The PPI dendrimers have also been

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modified at the exterior with certain groups to improve the gene delivery potency (Hao et al., 2009; Koppu et al., 2010; Taratula et al., 2009).

Poly(γ-benzyl-L-glutamate) (PBLG) is a derivative of poly L-glutamic acid which is extensively used for drug-sustained delivery carrier. After aminolysis, the PBLG can turn into polyglutamine that can be used as potential gene delivery vector (Chen et al., 2010) and the suitable aminolysis agents is a vital factor for preparing efficient gene vector. Pan synthesized PAMAM-PBLG, and polyamidoamine-poly N,N′-di-(2-aminoethyl) aminoethyl glutamine (PAMAM-PAGA) copolymer was prepared by aminolysis (Pan et al., 2013a,b). Zeng synthesized a biodegradable and pompon-like dendrimer using polyamidoamine (PAMAM) G4.0 as the inner core and low-molecular-weight polyethylenimine as the surrounding multiple arms (Zeng et al., 2011).

With the aim to create serum resistant, biodegradable and non-toxic transfection agents, we have recently developed the multi-armed dendrimers which were composed of PPI G2.0 as the inner core and poly(L-glutamic acid) PLG grafted low generation PPI(G1.0 or G2.0) as the surrounding multiple arms. The multi-armed poly (L-glutamic acid)-graft-PPI copolymers (MP-g-PPI) combined the merits of a well-defined globular structure of PPI G2.0 and the low-toxicity and biodegradable chains of PLG. Moreover, the low generation PPI was graft on the PLG chains can compact and protect DNA well. The physicochemical and biophysical properties of the MP-g-PPI have been explored such as DNA condensation, DNase protection as well as particle sizes and zeta potentials. Furthermore, the toxicity and transfection profiles of the dendrimers were especially investigated in the absence and presence of serum.

2. Materials and methods

2.1. Materials

Polyethylenimine 25 kDa (PEI 25 K), G1.0 PPI tetramine dendrimer (DAB-Am-4), 2-hydroxypyridine(2-HP), 3-[4,5-dimethylthiazol-2-yl]-2,5-diphe-nyl-tetrazolium bromide (MTT), 4′,6-diamidino-2-phenylindole (DAPI), Lipofectamine 2000 (Lipo-2K) were purchased from Sigma–Aldrich (USA). DNase I was purchased from Fermentas (USA). G2.0 PPI (DAB-Am-8) was provided by Shenzhen Institute of Advanced Technology, Chinese Academy of Sciences. ^{1}H NMR of G2.0PPI ($CDCl_3$, δ_{ppm}): 1.33 (m, 4H, —$NCH_2CH_2CH_2CH_2N$—), 1.55 (m, 24H, —$NCH_2CH_2CH_2N$—), 2.34 (m, 20H, —$NCH_2CH_2CH_2N$—), 2.39 (m, 16H, —$NCH_2CH_2CH_2NH_2$), 2.67 (t, 16H, —CH_2NH_2). N-carboxyanhydride of γ-benzyl-L-glutamate (BLG-NCA) was prepared according to Daly's method (Huang et al., 2007). ^{1}H NMR of BLG-NCA($CDCl_3$, δ_{ppm}): 2.05–2.25 (m, 2H, —CH_2CH_2COO—), 2.61 (m, 2H, —CH_2CH_2COO—), 4.37 (t, 1H, CH), 5.16 (s, 2H, $PhCH_2O$—), 7.32–7.40 (m, 5H, Ph). Methanol, N,N-dimethyl formamide (DMF), tetrahydrofuran (THF), petroleum ether (bp 30–60 °C), diethyl ether, chloroform were purchased from Guangzhou Reagent Inc. (Guangzhou China), which were distilled to remove any traces of water before use. Fetal bovine serum (FBS), Dulbecco's modified Eagle medium (DMEM) and RPMI 1640 culture medium were purchased from Invitrogen Gibco (USA). Label IT TM-rhodamine labeling kit and Lab-Tek Chambered coverglass system were purchased from Mirus (USA).

2.2. Cell lines

Hela cell line was obtained from Animal Center of Sun Yat-sen University (China). Hek-293 cell line was purchased from Shanghai Cell Center, Chinese Academy of Sciences (China).

2.3. Plasmid DNA

The reporter plasmids were pEGFP-C1 and pGL3-luc, kindly donated by Guang Dong Pharmaceutical University (Guangzhou, China). The plasmids were propagated in Escherichia coli DH5α and purified with a Qiagen kit (Chatsworth, USA).

2.4. Preparation of multi-armed PPI-PBLG (MP)

BLG-NCA was added into anhydrous $CHCl_3$ (1 g/100 mL) and stirred vigorously to dissolve. After BLG-NCA was completely dissolved in the reaction solution, G2.0PPI was added as the initiator at a monomer/initiator molar ratio of 100:1. The mixture solution was then stirred for 72 h at room temperature. After reaction, the solution was concentrated, and then precipitated with an excess of cold diethyl ether. After filtration, multi-armed MP was obtained by standing under vacuum at room temperature for 24 h (yield: 73%).

2.5. Synthesis of MP-g-PPI

Aminolysis of MP was performed according to the literature (Wen et al., 2009). MP was dissolved in dried DMF (1 g/10 mL), and then excess PPI and 2-HP were added. The mixture solution was stirred for 48 h at 40 °C. The detailed feeding amount is presented in Table 2. After reaction, the solution was dialysed (using tubing with molecular weight cut-off of 3500 Da) against distilled water (3 days) directly. The products were collected by freeze-drying finally (yield: 70% and 66% for MP-G1.0PPI, MP-G2.0PPI, respectively).

2.6. Characterization of MP-g-PPI polymers

FT-IR spectra of polymers were measured by Spectrum100 Fourier transformation infrared spectrometer (PerkinElmer, Germany). ^{1}H NMR spectra of polymers were determined by an AVANCE (600 MHz) NMR spectrometer (Bruker, Germany).

2.7. Self-assembly of MP-g-PPI/pDNA complexes

The MP-G1.0PPI/pEGFP-C1 or MP-G2.0PPI/pEGFP-C1complex was self-assembled by adding MP-G1.0PPI or MP-G2.0PPI solutions in deionized water to equal volumes of pEGFP-C1 solutions in deionized water. Each mixture was gentle vortexed and incubated at room temperature for 30 min. The concentration of the polymer solution was based on the weight ratio.

2.8. Agarose gel electrophoresis assays

The combination of MP-g-PPI with pDNA can be assayed by agarose gel electrophoresis. Firstly the MP-g-PPI/pDNA complexes at different weight ratios were freshly prepared and then electrophoresed on 1.0% (w/v) agarose gel containing 0.5 μg/mL ethidium bromide at 100 V for 50 min. The DNA bands of complexes were visualized on an UV illuminator and photographed using a GelDoc XR gel image system (Biorad, USA). Meanwhile the G1.0 PPI, G2.0 PPI and PEI 25 K complexes were used as controls.

2.9. Protection test of DNA from digestion of DNase I

The protection test of DNA from DNase I digestion was carried out according to the literature (Mimi et al., 2012). The prepared MP-g-PPI/pDNA complexes solution (100 μg/mL 10 μL) were incubated with 4 μL DNase I (100 μg/mL, in 50 mmol/L Tris–Cl, 10 mmol/L MgCl, pH 7.4) at 37 °C for 30 min. Then, 50 mM of EDTA was added to inactivate DNase I followed by incubation at 65 °C for 15 min. Finally,