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The characteristics and transfection efficiency of cationic poly (ester–co–urethane) – short chain PEI conjugates self-assembled with DNA

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ABSTRACT

To improve the transfection efficiency of polycations with DNA, we synthesized poly(ester–co–urethane)(PEU-g-PEI800) with short chain PEI800 in the side chain, and poly(ester–co–urethane)(PEU) without short chain PEI800. Both PEU-g-PEI800 and PEU, readily self-assembled with plasmid DNA (pCMV-βgal) in a HEPES buffer, were characterized by dynamic light scattering and zeta-potential. The results reveal that PEU-g-PEI800 and PEU were able to self-assemble particles with DNA and yield nano-sized complexes (<200 nm) with positive charge at N/P ratios of 20/1 and 120/1, respectively. The degradation studies indicate that the half-life of PEU-g-PEI800 and PEU in the HEPES buffer were 14 and 35 h at pH 7.4, respectively. Titration studies were performed to determine the buffering capacities of the polymers. The COS-7 cell viabilities in the presence of PEU-g-PEI800/DNA, PEU/DNA, and PEI25k/DNA were studied. In addition, The PEU-g-PEI800/DNA complexes were able to transfect COS-7 cells in vitro with a high efficiency comparable to a well-known gene carrier PEI25k. The results indicate that PEU-g-PEI800 is an attractive cationic poly (ester–co–urethane) for gene delivery and an interesting candidate for further study.

1. Introduction

Gene therapy requires a gene delivery system that is efficient and has no or low cytotoxic side effects. In polymer-based gene delivery systems, the complexes of condensed DNA with polycations have many advantages [1–3]. Cationic polymers not only condense DNA into nano-particle small enough to enter cell, but also protect negatively charged strands of DNA nuclease degradation. Additionally, cationic polymers would provide a pH-buffering capacity allowing them to behave as a proton sponge which would assist in the escape of complexes from lysosome and improve the transfection efficiency [4,5]. However, some amino-containing polymers, such as poly(2-(dimethylamino))ethyl methacrylate (PDMAEMA), poly(amido-amine)(PAA), and poly (ethylenimine)(PEI), demonstrate a considerable degree of cytotoxicity [6–9]. Consequently, there have been great efforts to synthesize biodegradable polycations that can be used as gene carriers. A number of reported biodegradable gene carriers including poly(4-hydroxy-L-proline ester) [10,11], poly(β-amino ester) [12–15], polyphosphoester [16], and polyurethane [17,18], have been synthesized. Polyesters are well-know biodegradable biomaterials used in wide range of applications, including the controlled release of DNA-based therapeutic agents. Polyurethanes, because of their biodegradability and functionality in terms of chemical and physical properties, have been investigated for different biomedical applications [19,20]. Polyester and polyurethanes are considered to be a biocompatible, biodegradable, and low-toxicity material with high cationic potential. However, these materials have a significant limitation, namely, low transfection efficiency. One of the primary causes of poor gene delivery is the inefficient release of vectors from endosomes into the cytoplasm. On the other hand, high-molecular-weight poly (ethylenimine) (PEI) has been revealed to be the most effective non-viral vector based on cationic polymers owing to its high pH-buffering capacity that is believed to enhance the exit of vectors from the endosomes compartment [21]. However, sometimes the high toxicity of high-molecular-weight PEI limits its application in gene therapy. In this study, amine-containing poly (ester–co–

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urethane) (PEU-g-PEI800) bearing short chain PEI800 in the side chain has been designed and synthesized and their structure in correlating to DNA condensation capacity, biodegradability, and cytotoxicity are discussed in this article.

2. Materials and methods

2.1. Materials

Glycidyl methacrylate and 1,4-diaminobutane were purchased from Acros Co. (USA). N,N-dimethylethylenediamine and n-hexane were obtained from Fluka Co. (Switzerland). The solvent of N,N-dimethylformamide (DMF, Tedia Co., USA) was dried over calcium hydride and distilled just before use. Polyethylenimine (Branched PEI, $M_w = 25{,}000$ and $M_w = 800$), 6-amino-1-hexanol, ethyl isocyanatoacetate, and N-[2-hydroxyethyl] piperazine-N′-[2-ethanesulfonic acid] (HEPES) were obtained from Sigma Co. (USA). N-methyldibenzopyrazine methyl sulfate (electron-coupling reagent) and sodium (2,3-bis(2-methoxy-4-nitro-5-sulphophenyl)-2H-tetrazolium-5-carboxanilide) (XTT) were purchased from Roche Co. (USA). The plasmid pCMV-LacZ (pCMV-βgal) contained a CMV promoter to drive the β-galactosidase (LacZ) gene expression [22,23]. The plasmid DNA was amplified in *Escherichia coli* (DH5α strain) and purified using column chromatography (Qiagen® Plasmid Mega kit, Germany). The purified plasmid DNA was dissolved in a tris(hydroxymethyl) methylamine-ethyldiaminetetraacetic acid (Tris–EDTA) buffer (pH 8.0) and determined using the ratio of UV absorbance at 260 nm/280 nm. Monkey SV40 transformed kidney fibroblast COS-7 cells were obtained from American Type Culture Collection (ATCC, CRL-1651). The cells were cultured in Dulbecco's modified Eagle's medium (DMEM, GibcoBRL Co., Ltd.) supplemented with 10% FBS, 4.5 g/L glucose, 1.5 g/L sodium bicarbonate, and 4 mM L-glutamine, and maintained at 37 °C in a humidified 5% CO_2-containing atmosphere.

2.2. Polymer characterizations

The structures of the polymers were characterized by nuclear magnetic resonance (NMR, Bruker AMX-400 spectrometer) and Fourier transform infrared (FT-IR, Mattson Galaxy Series 5000 spectroscope). The molecular weight and distribution of the polymer were determined by gel permeation chromatography analysis (GPC, Waters Model LC-2410) based on polystyrene standards in THF.

2.3. Synthesis of EOD

EOD was synthesized as shown in the first step of Scheme 1. 1.4-Diaminobutane and glycidyl methacrylate with a molar ratio of 2/1 (–NH_2/epoxy) were mixed in anhydrous dichloromethane in a three-necked reaction flask under a dry nitrogen purge and then cooled to 18 °C to react for 24 h. The product was purified through column chromatography using elute solvent (acetone/ethyl acetate). The structure of EOD was characterized by FT-IR, ^{1}H NMR, and ^{13}C NMR.

EOD. ^{1}H NMR(400 MHz, d_6-DMSO, ppm) δ: 1.18(4H, –NHCH$_2$CH$_2$CH$_2$CH$_2$NH–), 2.08(2H, –NHCH$_2$CH$_2$CH$_2$CH$_2$NH–), 2.15(6H, –C(CH$_3$)=CH$_2$), 2.60(4H, –NHCH$_2$CH$_2$-CH$_2$CH$_2$NH–, 2.80(4H, –NHCH$_2$CH(OH)–), 4.06(2H, –NHCH$_2$CH(OH)–), 4.59(2H, –CH$_2$OCO–), 5.66(2H, –C(CH$_3$)=CH$_a$Hb), 6.07(2H, –C(CH$_3$)=CH$_a$Hb). ^{13}C NMR-(400 MHz, d_6-DMSO, ppm) δ: 18.53 (–COO–CCH$_3$=CH$_2$), 30.71 (–CH$_2$CH$_2$NH–), 49.12 (–CH$_2$CH$_2$NH–), 49.23 (–NH–CH$_2$CHOH–), 57.54 (–CH$_2$–CHOH–CH$_2$–), 59.71 (–CHOH–CH$_2$–COO–), 125.58 (–CCH$_3$=CH$_2$), 136.15(–CCH$_3$=CH$_2$), 175.15 (–COO–).

2.4. Synthesis of PEOH

The monomers of EOD and 6-amino-1-hexanol with a –C=C/–NH_2 molar ratio of 1/1 were mixed in anhydrous N,N-dimethylformamide (DMF) in a three-necked reaction flask under a dry nitrogen purge and then heated to 85 °C to react for 8 h. The product was precipitated in anhydrous ethyl ether and vacuum-dried at 40 °C. The structure of PEOH was characterized by FT-IR, ^{1}H NMR, and ^{13}C NMR.

PEOH. ^{1}H NMR(400 MHz, d_6-DMSO, ppm) δ: 1.23–1.35(16H, –NCH$_2$CH$_2$CH$_2$-CH$_2$CH$_2$CH$_2$OH; –CH(CH$_3$)CH$_2$–; –NHCH$_2$CH$_2$CH$_2$CH$_2$NH–), 1.68(2H, –NCH$_2$CH$_2$-CH$_2$CH$_2$CH$_2$CH$_2$OH), 2.12(2H, –NHCH$_2$CH$_2$CH$_2$CH$_2$NH–), 2.41(2H, –NCH$_2$CH$_2$CH$_2$CH$_2$CH$_2$-CH$_2$OH), 2.56(4H, –NHCH$_2$CH$_2$CH$_2$CH$_2$NH–), 2.73(2H, –OCOCH(CH$_3$)–), 2.77(4H, –NHCH$_2$CH(OH)–), 3.59(2H, –NCH$_2$CH$_2$CH$_2$CH$_2$CH$_2$CH$_2$OH), 4.07(2H, –NHCH$_2$CH(OH)–), 4.56(4H, –CH(OH)CH$_2$OCO–). ^{13}C NMR(400 MHz, d_6-DMSO, ppm) δ: 19.16(–CHCH$_3$–CH$_2$–), 25.27(–N–CH$_2$CH$_2$CH$_2$CH$_2$CH$_2$CH$_2$OH), 27.02(–N–CH$_2$CH$_2$CH$_2$CH$_2$CH$_2$CH$_2$OH), 27.11-(–CH$_2$CH$_2$NH–), 32.51(–N–CH$_2$CH$_2$CH$_2$CH$_2$CH$_2$CH$_2$-OH), 32.77(–COO–CHCH$_3$–CH$_2$–N–), 33.01(–N–CH$_2$CH$_2$CH$_2$CH$_2$CH$_2$CH$_2$OH), 49.71(–CH$_2$CH$_2$NH–), 53.55(–NHCH$_2$CHOH–), 56.15(–N–CH$_2$CH$_2$–), 62.61(–CHCH$_3$CH$_2$N–), 63.87(–CH$_2$CH$_2$CH$_2$OH), 69.93(–NHCH$_2$-CHOHCH$_2$COO–), 172.53(–COO–).

2.5. Synthesis of PEU

The polymer PEOH and ethyl isocyanatoacetate with a –OH/–NCO molar ratio of 1/1 were mixed in anhydrous N,N-dimethylformamide (DMF) in a three-necked reaction flask under a dry nitrogen purge and then heated to 45 °C to react for 24 h. The product was precipitated in anhydrous ethyl ether and vacuum-dried at 40 °C. The structure of PEU was characterized by FT-IR, ^{1}H NMR, and ^{13}C NMR.

PEU. ^{1}H NMR(400 MHz, d_6-DMSO, ppm) δ:1.23–1.39(16H, –NCH$_2$CH$_2$CH$_2$-CH$_2$CH$_2$CH$_2$OCO–; –CH(CH$_3$)CH$_2$–; –NHCH$_2$CH$_2$CH$_2$CH$_2$NH–), 1.62(2H, –NCH$_2$CH$_2$-CH$_2$CH$_2$CH$_2$CH$_2$OCO–), 2.10(2H, –NHCH$_2$CH$_2$CH$_2$CH$_2$NH–), 2.36(2H, –NCH$_2$CH$_2$-CH$_2$CH$_2$CH$_2$CH$_2$OCO–), 2.53(4H, –NHCH$_2$CH$_2$CH$_2$CH$_2$NH–), 2.73(2H, –OCOCH(CH$_3$)–), 2.77(4H, –NHCH$_2$CH(OCO–)CH$_2$–), 3.71(6H, –OCONHCH$_2$CO–), 3.97(12H, –CH$_2$OCO–; –OCOCH$_2$CH$_3$), 4.36(2H, –NHCH$_2$CH(OCO)–) 4.69(4H, –CH(OCO–)CH$_2$OCO–). ^{13}C NMR(400 MHz, d_6-DMSO, ppm) δ: 18.25(–CHCH$_3$–CH$_2$–), 24.21(–N–CH$_2$CH$_2$-CH$_2$CH$_2$CH$_2$CH$_2$O–), 25.32(–CH$_2$CHCH$_3$–COO–), 26.61(–N–CH$_2$CH$_2$CH$_2$CH$_2$–), 28.12(–CH$_2$CH$_2$NH–), 33.72(–CH$_2$CH$_2$CH$_2$O–), 33.73(–CH$_2$CH$_2$CH$_2$–O–), 35.74(–CHCH$_3$-CH$_2$–N–), 43.81(–COO–NH–CH$_2$–COO–), 45.42(–CH$_2$CH$_2$NH–), 52.77(–CH(O)CH$_2$–NH–), 58.23(–N–CH$_2$CH$_2$–), 58.28(–NHCH$_2$COOCH$_2$CH$_3$), 63.11(–CH$_2$OCOCH$_2$CH$_3$), 63.23(–CHCH$_3$CH$_2$N–), 63.51(–CH(O)CH$_2$–COO–), 64.11(–NHCH$_2$CH(O)CH$_2$–), 157.62(–COONHCH$_2$–), 170.41(–CH$_2$COOCH$_2$CH$_3$), 171.53(–CH$_2$CH(CH$_3$)–COO–).

2.6. Synthesis of PEU-g-PEI800

The polymer PEU and polyethylenimine(PEI800) with a –OCOCH$_2$CH$_3$/PEI molar ratio of 1/1 were mixed in anhydrous N,N-dimethylformamide (DMF) in a three-necked reaction flask under a dry nitrogen purge and then heated to 50 °C to react for 48 h. The product was precipitated in anhydrous ethyl ether and vacuum-dried at 40 °C. The structure of PEU-g-PEI800 was characterized by FT-IR, ^{1}H NMR, and ^{13}C NMR.

PEU-g-PEI800. ^{1}H NMR(400 MHz, d_6-DMSO, ppm) δ:1.23–1.39((16H, –NCH$_2$CH$_2$CH$_2$CH$_2$CH$_2$OCO–; –CH(CH$_3$)CH$_2$–; –NHCH$_2$CH$_2$CH$_2$CH$_2$NH–), 1.60(2H, –NCH$_2$CH$_2$CH$_2$CH$_2$CH$_2$OCO–), 2.10(2H, –NHCH$_2$CH$_2$CH$_2$CH$_2$NH–), 3.83(6H, –OCO NHCH$_2$CO–), 3.97(6H, –CH$_2$OCO–), 4.36(2H, –NHCH$_2$CH(OCO)–), 4.69(4H, –CH(OCO–)CH$_2$OCO–). ^{13}C NMR(400 MHz, d_6-DMSO, ppm) δ: 18.92(–CHCH$_3$–CH$_2$–), 25.23(–N–CH$_2$CH$_2$CH$_2$CH$_2$CH$_2$O–), 25.68(–CH$_2$CHCH$_3$–COO–), 28.10(–N–CH$_2$CH$_2$CH$_2$CH$_2$–), 28.53(–CH$_2$CH$_2$NH–), 32.59(–CH$_2$CH$_2$CH$_2$O–), 32.64(–CH$_2$)$_4$CH$_2$CH$_2$OCO–), 35.73 (–CHCH$_3$–CH$_2$–N–), 44.13(–COONHCH$_2$COO–), 46.38(–CH$_2$CH$_2$NH–), 54.42(–NHCH$_2$CH(–O–)CH$_2$–), 55.17(–N–CH$_2$CH$_2$–), 60.63(–N(CH$_2$)$_5$CH$_2$OCO–), 60.72(–CHCH$_3$CH$_2$N–), 64.36(–CH(O)CH$_2$–COO–), 64.52(–CH(O)CH$_2$–COO–), 66.12(–CH$_2$CH(–O–)CH$_2$OCO–), 157.12(–COONHCH$_2$–), 170.53(–CH$_2$CONH–), 171.55(–CH$_2$CH(CH$_3$)–COO–).

2.7. Acid–base titration assay of polymers

Acid–base titration was used to evaluate the buffering capacity of synthesized cationic polymers. In this assay, 10 mg of PEU-g-PEI800 was dissolved in 10 mL of 150 mM NaCl. 100 μL of 1 N NaOH was then added to the solution to adjust the pH to 11.6 before it was titrated with acid. The solution was titrated with increasing volumes of 0.1 N HCl solutions, and the results were measured using a pH meter. The pH range of PEU was determined using the same procedure.

2.8. Hydrolytic degradation of polymers

PEU-g-PEI800 and PEU were dissolved in a buffer solution (pH 7.4) with a concentration of 10 mg/mL, and then incubated in a water bath at 37 °C for various durations. After hydrolysis for various durations, the solution was dried in a vacuum for several hours to remove water. The molecular weight of the polymer was determined using gel permeation chromatography (GPC).

2.9. XTT assay

The influence of the polymer concentration on the cell viability was evaluated in a cell culture for the various polymers. The cytotoxicities of PEU-g-PEI800/DNA and PEU/DNA for comparison with that of PEI25k/DNA were evaluated using the XTT assay. In a 96-well plate, COS-7 cells were cultured in complete DMEM and then seeded at a density of 1.0×10^4 cells/well. The cells were incubated at 37 °C and 5% CO_2 in a humidified atmosphere for 24 h. Subsequently, the cells were incubated for one hour in 200 μL FBS-free DMEM containing polymer with various concentrations. The cells were incubated in DMEM as a negative control. After 1 h, the cells were washed with 200 μL PBS solution and replaced by complete DMEM for a further 48 h of incubation. Then, 50 μL of XTT labeling mixture was added to each well and the cells were further incubated at 37 °C for 1 h. Results are expressed as the relative cell viability (%) with respect to control wells containing culture medium.

2.10. Formation of polymer/DNA complexes

10.0 mg/mL of the polymer was dissolved in 20 mM HEPES buffer (pH 7.4) and its serial dilutions were made with the various mass ratios of polymers/DNA (w/w). The complexes were then allowed to self-assemble in the HEPES buffer. Then incubated at room temperature for 30 min before measurements.

2.11. Characterizations of polymer/DNA complexes

The particle sizes and surface charges of the polymer/DNA complexes were determined by dynamic light scattering (Nicomp 380 system, USA) and