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Glycopolymer modification on physicochemical and biological properties of poly(L-lysine) for gene delivery

Dezhong Zhou[a], Congxin Li[a], Yuling Hu[a], Hao Zhou[b], Jiatong Chen[b], Zhengpu Zhang[a], Tianying Guo[a,*]

[a] Key Laboratory of Functional Polymer Materials (Nankai University), Ministry of Education, Institute of Polymer Chemistry, College of Chemistry, Tianjin, China
[b] Department of Biochemistry and Molecular Biology, College of Life Science, Nankai University, Weijin Road, No. 94, Tianjin 300071, China

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ABSTRACT

Poly(L-lysine) (PLL) has excellent plasmid DNA (pDNA) condensation capacity. However, the relatively high cytotoxicity and low transfection efficiency limit its application as gene delivery vectors. Here, well-defined glycopolymers are synthesized by reversible addition fragmentation transfer polymerization and grafted onto PLL to improve the gene delivery performance. After glycopolymer modification, PLL shows reduced cytotoxicity. By regulating the glycopolymer length and amino group substitution degree, the glycopolymer modified PLL can condense pDNA with proper strength, protect the condensed pDNA from degradation and release them in time. Transfection with NIH3T3 and HepG2 cells shows that the glycopolymer modified PLL has improved transfection efficiencies. The low cytotoxicity, effective pDNA protection and enhanced transfection efficiencies indicate that glycopolymer modification would be an effective strategy to improve the polycation properties for gene delivery.

1. Introduction

Over the past two decades, gene therapy has attracted much attention [1]. However, how to design safe and efficient gene delivery vectors is one of the bottlenecks for successful gene therapy [2]. An ideal vector should be (1) safe with low cytotoxicity, (2) of high gene transfer efficiency and (3) specific to the target tissues [3]. Gene delivery vectors are divided into viral and non-viral carries. Based on the safety consideration, non-viral vectors have been greatly developed. Natural polycations, such as chitosan, have drawn much attention [4]. As a cationic peptide, poly(L-lysine) (PLL) is favor of interacting with plasmid DNA (pDNA) due to the high positive charge density. However, the application of PLL as gene delivery vector is limited because of the high cytotoxicities [5,6] and low gene transfection efficiencies [7,8]. In order to improve the gene delivery efficiency of the PLL, many attempts have been made. For example, introducing polyethylene glycol (PEG) to reduce the cytotoxicity [9], grafting histidine derivatives to improve the polycations/pDNA endosomal escape [10] or tethering target ligands to promoting complex cellular uptake [11,12]. Nevertheless, comparing with other polycations, such as chitosan (CS), poly(ethylene imine) (PEI) and poly(amido amine) (PAMAM), the applications of PLL are widely limited [13–15].

As a cationic polyelectrolyte, the cytotoxicity and gene transfection efficiencies of PLL are relevant to the polycationic properties in biofluid. Saccharides are abundant natural building blocks used to construct many important biological polymers including proteins, polysaccharides and glycoproteins [16,17]. Herein, we attempted to regulate the PLL polyelectrolyte properties by grafting with well-defined saccharide-containing polymers (glycopolymers). Influences of the glycopolymer length, amino group substitution degree (SD) on the cytotoxicity and pDNA condensation capacity of the modified PLL, polycations/pDNA complex stability and the corresponding gene transfection efficiencies to NIH3T3 and HepG2 cells are investigated.

2. Experimental

2.1. Materials

Poly(L-lysine) (PLL, M_w = 150–300 kDa), agarose, trypsin–EDTA, Dulbecco's modified Eagle medium (DMEM), heparin, deoxyribonuclease I (DNase), ethidium bromide (EB), fetal bovine serum (FBS), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazoliumerma bromide (MTT) and phosphate buffer solution (PBS, 0.01 M, pH = 7.2–7.4) were obtained from Beijing Dingguo Biotech. Co. Ltd. (Tianjin, China). 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC·HCl, 98.5%) and *N*-hydroxysuccinimide (NHS, analytical grade) were purchased from Aladdin (Shanghai, China). 2,2′-Azobisisobutyronitrile (AIBN) got from Sigma–Aldrich (Shanghai, China) was recrystallized prior to use. Plasmid

* Corresponding author. Tel.: +86 22 235 015 97; fax: +86 22 235 015 97.
E-mail address: tyguo@nankai.edu.cn (T. Guo).

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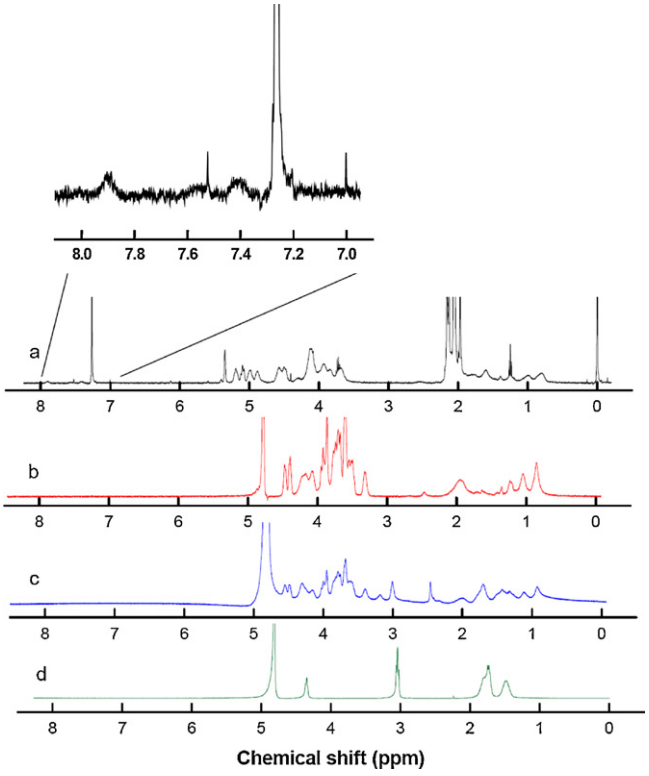

Fig. 1. ^{1}H NMR spectra of (a) PMAEL-1 in $CDCl_3$, (b) DPMAEL-1 in D_2O, (c) DPMAEL-g-PLL-1 in D_2O, (d) p-PLL in D_2O.

pEGFP-N1 (4.7 kbp; Clontech, Palo Alto, CA, USA) encoding enhanced green fluorescent protein (EGFP) was driven by immediate early promoter of CMV. The plasmid DNA (pDNA) was maintained, propagated in DH5α strain of *E. coli* and purified by using of Endfree plasmid kit (Tiangen, China). The purity and concentration were confirmed by spectrophotometry (A_{260}/A_{280}). Chain transfer agent (CTA) 4-cyanopentanoic acid dithiobenzoate (CPADB) was synthesized according to reference [18]. Glycomonomer 2-*O*-meth-acryloyloxyethoxyl-(2,3,4,6-tetra-*O*-acetyl-*β*-D-galactopyranosyl)-(1-4)-2,3,6-tri-*O*-acetyl-*β*-D-glucopyranoside (MAEL) was synthesized according to our previous work [19].

2.2. Preparation and characterization

2.2.1. Preparation of the glycopolymers and glycopolymer modified PLL

The glycopolymers were synthesized by RAFT polymerization with CPADB as the CTA. For the preparation of the relatively shorter glycopolymer PMAEL-1, the molar ratio of monomer, CTA and initiator was set as: $[MAEL]_0:[CPADB]_0:[AIBN]_0 = 60:2:1$. Briefly, MAEL (2.0 g), CPADB (0.025 g) and AIBN (0.007 g) were dissolved in $CHCl_3$ (20 mL). After degassed by three freeze–pump–thaw cycles, polymerization was conducted in a water bath at 70 °C for 24 h under N_2 atmosphere. When the polymerization was completed, the reaction was quenched with ice water. The product was precipitated by cold diethyl ether. After three times purification by the dissolution–precipitation method, the final product was dried under vacuum for 24 h. Under the same conditions, PMAEL-2, the relatively longer glycopolymer, was synthesized just by adjusting the molar ratio of monomer, CTA and initiator to be: $[MAEL]_0:[CPADB]_0:[AIBN]_0 = 240:2:1$.

Then, the glycopolymers were deprotected. Briefly, PMAEL-1 or PMAEL-2 was dispersed in anhydrous methanol; sodium methoxide (30%, mass concentration) was added and the mixture was stirred for 1 h at room temperature. Next, the suspension was isolated by centrifugation and dialyzed with deionized water for 3 days (cutoff M_w = 3.5 kDa) and lyophilized, the obtained deprotected glycopolymers were denoted as DPMAEL-1 and DPMAEL-2.

For fabricating glycopolymer modified PLL, DPMAEL was dissolved in distilled water, EDC and NHS (1:1 in molar ratio) were added and stirred for 1 h, then PLL was added. The solution was further stirred for 48 h. Afterwards, the solution was dialyzed with deionized water for 3 days (cutoff M_w = 12–14 kDa) and lyophilized.

Table 1
Molecular weight and polydispersity of glycopolymers.

Glycopolymer	M_n	M_w	PDI
PMAEL-1	5416	6201	1.14
PMAEL-2	20,059	24,623	1.22

Table 2
DPMALE-g-PLL conjugate composition.

Conjugate	Glycopolymer	SD (%)
DPMAEL-g-PLL-1	DPMAEL-1	9.4
DPMAEL-g-PLL-2	DPMAEL-1	36
DPMAEL-g-PLL-3	DPMAEL-2	5.2
DPMAEL-g-PLL-4	DPMAEL-2	17

2.2.2. Characterization

The molecular weight (M_w) and polydispersity (PDI) of the PMAEL-1 and PMAEL-2 were determined with gel permeation chromatograph (GPC) equipped with Waters 2414 refractive index detector and Waters 1525 Binary HPLC Pump, using Waters Styragel HT2, HT3, HT4 THF 7.8 mm × 300 mm columns. Calibration was based on low polydispersity Shodex polystyrene standards. THF was used as the eluent at a flow rate of 1.0 mL/min operated at 35 °C.

The chemical structure of the glycopolymers and the glycopolymer modified PLL were characterized with proton magnetic resonance spectra (^{1}H NMR) on a Varian UNITY-plus 400 spectrometer operated at 400 MHz.

pDNA condensation by DPMAEL-g-PLL and PLL was evaluated by agarose gel electrophoresis in Tris–boric acid–EDTA buffer (pH = 8.0). In general, pDNA employed for each sample was fixed at 120 ng, according to the N/P ratio (charge ratio of amine to phosphate), the required DPMAEL-g-PLL or p-PLL solution was dropped into the pDNA solution and the mixture was mixed. 30 min or 24 h later, bromophenol blue-containing sucrose solution was added and the whole mixture volume was adjusted to 25 μL with PBS. Then the mixture was transferred to the agarose gel well, electrophoresis was conducted at 100 V for 45 min. Afterwards, the agarose gel was stained by EB (0.5 μg/mL) for 20 min and the pDNA migration patterns were revealed by UV irradiation.

The disassociation behaviors of the polycations/pDNA complexes were evaluated by the heparin replacement experiment [20]. After the complex preparation, heparin (135 USP units per 1 μg pDNA) and NaCl (5 M, 1 μL) were added and the mixture was incubated at 4 °C for 30 min. As controls, the same samples without heparin addition were treated in an analogous manner. Then samples were treated as above.

For testing the stability of the polycations/pDNA complexes in the presence of DNase, after complex preparation, $MgCl_2$ (2.5 mM final concentration) and DNase (2 USP units per 1 μg pDNA) were added. The mixture was incubated at 37 °C for 10 min, then the enzyme reaction was stopped by EDTA (15 mM final concentration) and the samples were transferred onto ice. As control, 120 ng naked pDNA was digested with DNase. The samples were analyzed as described above.