Biomaterials 30 (2009) 4824–4832

Contents lists available at ScienceDirect

Biomaterials

journal homepage: www.elsevier.com/locate/biomaterials

Polyethylenimine-grafted polycarbonates as biodegradable polycations for gene delivery

Chang-Fang Wang, Yan-Xin Lin, Tao Jiang, Feng He*, Ren-Xi Zhuo

Key Laboratory of Biomedical Polymers of Ministry of Education, Department of Chemistry, Wuhan University, Wuhan 430072, China

A R T I C L E I N F O

Article history:
Received 27 March 2009
Accepted 19 May 2009
Available online 17 June 2009

Keywords:
Gene delivery
PEI-grafted
Biodegradable polycarbonates
Enzymatic ring-opening polymerization
Cytotoxicity
Transfection

A B S T R A C T

Polycations as one of non-viral vectors have gained increasing attentions. In this paper, polyethylenimine(PEI)-grafted polycarbonates (PMAC-*g*-PEI*x*) were synthesized as a kind of biodegradable polycations for gene delivery. Backbone polymer, poly(5-methyl-5-allyloxycarbonyl-trimethylene carbonate) (PMAC), was synthesized in bulk catalyzed by immobilized porcine pancreas lipase (IPPL). Then, PMAC–O, the allyl epoxidation product of PMAC, was further modified by PEI*x* with low molecular weight ($x = 423$, 800 and 1800). The MWs of PMAC-*g*-PEI*x*, measured by GPC–MALLS, were 81,900, 17,9900 and 200,600 g/mol with polydispersities of 1.2, 1.4 and 1.7, respectively. PMAC-*g*-PEI*x* could form positively charged nano-sized particles (30–90 nm) with *p*DNA, and all the three PAMC-*g*-PEI*x*/DNA polyplexes had similar buffer capabilities. *In vitro* experiments demonstrated that the PAMC-*g*-PEI*x* showed much low cytotoxicity and enhanced transfection efficiency could be found in comparison with PEI25K in 293T cells. Furthermore, pre-incubation of PMAC-*g*-PEI1800 showed a weakening binding capacity with DNA. The biodegradability of PMAC-*g*-PEI*x* can facilitate the efficient release of *p*DNA from polyplexes and reduce cell cytotoxicity. These results suggested that PMAC-*g*-PEI*x* would be a promising non-viral biodegradable vector for gene delivery system.

© 2009 Elsevier Ltd. All rights reserved.

1. Introduction

Gene therapy has been attracting more attentions for the treatment of genetic and acquired diseases that are currently incurable, such as immunodeficiency [1], cystic fibrosis [2], and Parkinson's disease [3]. In response, two primary gene vehicle classes have been developed: recombinant viruses and non-viral vectors [4]. The former has high transfection efficiency for both DNA and RNA to numerous cell lines [5], but including toxicity, immunogenicity, combining limited molecular DNA and hard for large-scale preparation acceptable for clinical testing [6]. Alternatively, non-viral vectors, such as liposomes, cationic polymers, dendrimers, nanoparticles and so on, show many advantages including less immune reaction to tissue, control of the structure of molecule, cell/tissue targeting, fitting various plasmid sizes, low cost and easy to synthesis [7].

Among non-viral systems, polycations are proposed because it is easy to form self-assembly with DNA [7]. Polyethylenimine (PEI) has been one of the most popularly and powerfully employed cationic gene carriers. High molecular weight PEI, such as PEI25K, shows a high transfection efficiency but also a high cell cytotoxicity. Low molecular weight PEI (Mw $\leq$ 2000), by contrast, has demonstrated a low cytotoxicity but also cannot be used as gene vectors due to rather unsatisfactory transfection efficiency. Therefore, modifying low molecular weight PEI as gene carriers are extensively investigated now, such as Michael addition of PEI*x* ($x = 600$, 1200 and 1800) with polycaprolactone diacrylate [8], cyclodextrin (CD)-modified PEI derivatives [9], dextran-hexamethylenediisocyanate grafted PEIs [10], dithiobis(succinimidylpropionate) or dimethyl-3,3′-dithiobispropionimidate modified low molecular weight PEI [11]. The transfection efficiency of these materials was comparable to that of PEI25K and the cytotoxicity was reduced to some degree [8–11]. The results showed that the charge density should not be too high which may combine membrane and destroy the cells metabolism, while the degradability of polycations would benefit to decrease the cytotoxicity. However, it remains challenging to obtain efficient gene vectors by careful molecular design, which combine the advantages of PEI25K with the low cytotoxicity of the low molecular weight PEIs.

Aliphatic polycarbonates are one of the important biodegradable polymers with good biocompatibility, favorable mechanical properties, low toxicity and biodegradability [12]. The properties of aliphatic polycarbonates could be further modified and designed by introducing pendant functional groups, which can be used to not

* Corresponding author. Tel.: +86 27 6875 4061; fax: +86 27 6875 4509.
E-mail addresses: fenghe2002@hotmail.com, hefeng@whu.edu.cn (F. He).

0142-9612/$ – see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.biomaterials.2009.05.053

only adjust the properties of polycarbonates but also facilitate further modification. Recently, aliphatic polycarbonates with pendant functional groups have been synthesized by ring-opening polymerization (ROP), such as hydroxyl [13], amino [14], allyl [15], carboxyl [16], etc. The synthesis of novel cyclic monomers containing a variety of functionalities has become an important method for the development of new biodegradable polymer materials with special properties for different applications.

Tin(II) 2-ethylhexanoate, which has been approved for surgical and pharmacological applications by the FDA, is generally employed as the catalyst for the synthesis of biomedical polymers. However, it has been reported that tin(II) 2-ethylhexanoate cannot be removed by a purification process such as the dissolution/precipitation method; thus, the residual Sn may be concentrated within matrix remnants after hydrolytic degradation [17]. To avoid the potential harmful effects of metallic residues in biomedical polymer materials, enzymatic polymerization is one of the powerful candidates for polymer synthesis [18]. Up to now, various kinds of biodegradable polymers have been synthesized by enzymatic ring-opening polymerization, such as polyesters and polycarbonates [18–20]. In our previous studies, we have reported that immobilized *porcine pancreas lipase* (IPPL) on silica particles can effectively catalyze the ring opening (co)polymerization of different six-membered cyclic carbonates [20,21]. The stability and recyclability of native enzyme can be improved significantly by immobilization [22].

In this paper, a biodegradable polycation, PMAC-*g*-PEI*x*, is presented as a non-viral gene vector. Grafted PEI residues were introduced to form stable polyplexes with *p*DNA due to their strong positive charges, while backbone polycarbonates was employed to reduce the cytotoxicity by controlling the charge density and preventing from combining cell membrane with its hydrophobic property. And also the biodegradability of polycarbonates would benefit to release DNA easily. Physicochemical properties of this vector were characterized and the DNA loading was evaluated.

2. Materials and methods

2.1. Materials

MAC was synthesized according to the literature [23,24] and recrystallized for several times before use. 3-Chloroperoxybenzoic acid (*m*CPBA, 70–75% water) was purchased from Sigma–Aldrich Co. and used as-received. PEI*x* ($x = 423$, 800 and 25K) and PEI1800 were purchased from Sigma–Aldrich Co. and Alfa Co., respectively. IPPL was prepared according to He et al. [21]. 3-[4,5-Dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT), Dulbecco's phosphate buffered saline (PBS), 3-Dulbecco's Modified Eagle's Medium (DMEM), fetal bovine serum (FBS) and trypsin were purchased from Invitrogen Corp. GelRed™ was purchased from Biotium (CA, USA). The human embryonic kidney transformed (293T) cells were incubated in DMEM containing 10% FBS and 1% antibiotics (penicillin–streptomycin, 10,000 U/mL) at 37 °C and a humidified atmosphere containing 5% CO_2. Other solvents were of analytical grade and purified by general methods.

PGL-3 and pEGFP-C1 plasmids were used as the reporter gene in this study. PGL-3 plasmid as the luciferase reporter gene was transformed in *Escherichia coli* JM109. pEGFP-C1 plasmid as the green fluorescent protein gene was transformed in *E. coli* DH5a. Both plasmids were amplified in Luria-Bertani (LB) medium at 37 °C overnight at 250 rpm. Then the purified plasmids as described by means of EndoFree were diluted by TE buffer solution and stored at −20 °C. The integrity of plasmids was identified by agarose gel electrophoresis. Its purity and concentration were determined by ultraviolet (UV) absorbance at 260 and 280 nm.

2.2. Synthesis of PMAC-g-PEIx

2.2.1. Synthesis of PMAC by enzymatic ring-opening polymerization

The vessel containing MAC and IPPL (0.2% w/w) with a magnetic stirring bar was sealed and dried with anhydrous phosphorus pentoxide as desiccant in vacuum. Then the vessel was placed in an oil bath at 80 °C for 2 h. The reaction mixture was dissolved in dichloromethane and the insoluble IPPL was removed by filtration. The solvent was condensed and poured into methanol to precipitate the polymer. The resulting polymer was dried in vacuo (yield: 86%).

2.2.2. Allyl epoxidation of PMAC

PMAC (0.10 g), 4 molar excess of *m*CPBA (70–75% water) and 5 mL $CHCl_3$ were charged into a flask with a magnetic stirring bar. The reaction mixture was refluxed for 12 h. Then the solution was filtered and precipitated into methanol twice. The obtained polymer (PMAC–O) was dried in vacuo (yield: 72%).

2.2.3. Synthesis of PMAC-g-PEIx

PMAC–O (0.10 g), 10 molar excess of PEI*x* ($x = 423$, 800 and 1800) and 20 mL $CHCl_3$ with a magnetic stirring bar in a flask was placed in oil bath to reflux for 2 h. The mixture was condensed and 1 M HCl aqueous solution was added. Then the neutralized products were dialyzed against distilled water (MWCO: 3500) at 4 °C for 24 h. Then the solution was lyophilized for 2 days to obtain the PEI-grafted polycarbonates (PMAC-*g*-PEI*x*).

2.3. Characterization

^{1}H NMR spectra were performed on a Mercury VX-300 spectrometer using $CDCl_3$ or D_2O–DMSO as the solvent. Gel permeation chromatography (GPC) measurements were detected on Waters-2690D HPLC and a 2410 refractive index detector. The eluent was chloroform with a flow rate of 1.0 mL/min. The narrow molecular weight distribution polystyrene was the standard. Gel permeation chromatography with multi angle laser light scattering (GPC–MALLS) was operated using Waters-2690D HPLC equipped with Ultrahydrogel 250 and 2000 columns (temperature 40 °C), and a Malls device (DAWN EOS, Wyatt Technology) as the index detector. Ultrapure water (20% acetonitrile, weight ratio) was used as eluent. Flow rate was 1.0 mL/min.

2.4. MTT cytotoxicity assay in vitro

The cytotoxicity of the resulting polycations was evaluated by MTT assay in 293T cells using PEI25K as the control. Generally, the 293T cells were seeded in a 96-well tissue culture plate at a density of 6000 cells/well in 100 μL DMEM medium containing 10% FBS for 24 h, and were exposed to the polyplex solutions at a series of concentration for another 48 h. Then, 20 μL MTT (5 mg/mL) solutions was added to each well and further incubated for 4 h. After that, the medium was exchanged by 150 μL DMSO to dissolve the formazan crystal formed by proliferating cells. The concentration of the proliferating cells in each well was confirmed by the absorbance of solvent at 570 nm using a microplate reader (Bio-Rad, Model 550, USA). The relative cell viability (%) was calculated according to the following:

$$\text{Cell viability } (\%) = (A_{\text{sample}}/A_{\text{control}}) \times 100,$$

where A_{sample} was obtained in the presence of polymers and A_{control} was obtained in the absence of polymers. The results were presented as the average values of four runs.

2.5. Agarose gel electrophoresis

*p*DNA condensing ability of PMAC-*g*-PEI*x* was examined by agarose gel electrophoresis. PMAC-*g*-PEI*x*/DNA polyplexes at various weight ratios ranging from 0.1 to 1.75 were prepared by adding appropriate volumes of polymer solution (2 mg/mL) to 1.3 μL of pGL-3 DNA (77 ng/μL in 40 mM Tris–HCl buffer solution). The volumes of the polyplexes were made up to a total volume of 6 μL with 150 mM NaCl solution. The polyplexes were then incubated at room temperature for 30 min, and were loaded on agarose gel (0.7%, w/v) containing GelRed and with Tris–acetate (TAE) running buffer at 80 V for 80 min. The location of *p*DNA bands was visualized with a UV lamp using a Vilber Lourmat imaging system (France).

2.6. Zeta potential measurements

Zeta potential measurements of polyplexes were carried out using a Nano-ZS 3600 (Malvern Instruments, USA) with a He–Ne Laser beam (633 nm, fixed scattering angle of 90°) at 25 °C. PMAC-*g*-PEI*x*/DNA polyplexes at weight ratios ranging from 2.5 to 160 were prepared by the same method with above agarose gel electrophoresis. After 30 min incubation in 100 μL ultrapure water, polyplex solutions were diluted to final volume of 1 mL before measurements.

2.7. Morphology of PMAC-g-PEIx/DNA polyplexes

PMAC-*g*-PEI*x*/DNA polyplexes were prepared at weight ratios of 10 and 60. Then the morphologies of the resulting polyplexes were observed by JEOL JEM-100CXII transmission electron microscope (TEM). The samples were prepared by dropping materials solution onto the copper grid with Formvar film and dried at 30 °C.

2.8. Acid–base titration

The buffer capability of PEI25K, PEI*x* and PMAC-*g*-PEI*x* was tested by acid–base titration assay over the pH values ranging from 10.00 to 2.00 described by previous reports [25]. Briefly, 0.2 mg/mL of each sample solution was prepared in 30 mL 150 mM NaCl solution. The pH value of the sample solution was adjusted to 10 by

Download English Version:

https://daneshyari.com/en/article/10230089

Download Persian Version:

https://daneshyari.com/article/10230089

Daneshyari.com