Journal of Controlled Release 128 (2008) 171–178

Contents lists available at ScienceDirect

Journal of Controlled Release

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

Synthesis of (Dex-HMDI)-g-PEIs as effective and low cytotoxic nonviral gene vectors

Yun-Xia Sun, Wang Xiao, Si-Xue Cheng, Xian-Zheng Zhang *, Ren-Xi Zhuo *

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

ARTICLE INFO

Article history:
Received 10 September 2007
Accepted 3 March 2008
Available online 12 March 2008

Keywords:
Polyethylenimine
Functionalized dextran
Gene vector

ABSTRACT

Gene vectors, (dextran-hexamethylenediisocyanate)-g-polyethylenimines ((Dex-HMDI)-g-PEIs), were synthesized through grafting low molecular weight (800 Da) branched polyethylenimine (PEI) to HMDI functionalized dextrans with two different molecular weights. The buffer capabilities of (Dex-HMDI)-g-PEIs were examined by acid–base titration. The titration profiles show that both (Dex-HMDI)-g-PEIs have the similar buffer capability regardless of the different molecular weight of dextran. Physiochemical characteristics of (Dex-HMDI)-g-PEI/DNA complexes were analyzed by agarose gel electrophoresis, and particle size and ζ-potential measurements. The result of gel electrophoresis suggests that both (Dex-HMDI)-g-PEIs are able to condense DNA efficiently at N/P ratios higher than 4. The particle sizes of (Dex-HMDI)-g-PEI/DNA complexes are around 160–250 nm, and the surface charges are around 19–23 mV at the N/P ratios ranging from 10 to 60. The morphology of complexes was observed by scanning electron microscopy (SEM) and the images show that nano-sized complexes display a regular spherical shape. In vitro cell viability and transfection were evaluated in 293T and HeLa cells using 25 kDa PEI as a control. The cytotoxicity of (Dex-HMDI)-g-PEIs is lower than that of 25 kDa PEI. The gene transfection efficiency of (Dex-HMDI)-g-PEI/DNA complexes at certain N/P ratios in 293T cells is higher than or comparable to 25 kDa PEI/DNA complex at its optimal N/P ratio of 10. In addition, comparing with (Dex-HMDI)-g-PEI with a high molecular weight dextran, (Dex-HMDI)-g-PEI with a low molecular weight dextran demonstrates lower cytotoxicity and higher transfection efficiency.

1. Introduction

Gene therapy is to cure the inherited or acquired diseases through delivering normal genes to targeted cells and replacing the unorderly genes [1,2]. In gene therapy, if naked DNA is injected to targeted tissues or cells directly, the efficacy is lower due to biodegradation and clearance of DNA and the low level of cellular uptake of naked DNA. To improve the transfection efficacy, gene vectors are necessary to protect genes from extracelluar nuclease degradation and facilitate the cell internalization. In general, gene vectors include viral and nonviral vectors. Viral vectors are limited due to the carcinogenesis and system immune response even though they have high efficiency [3,4]. Among nonviral vectors, polycations have attracted more attention due to the low immune response, simplicity for preparation, tissue or cells targeting and adjustable structures [4–6].

Polyethylenimines (PEIs) are a class of cationic polymers which have been proven to be effective nonviral vectors [7,8] due to their proton sponge effect which can buffer the environment of endosome and cause the release of the complex into the cytoplasm [9]. Then DNA gets accumulated in the nucleus of the targeted cells where the gene is expressed efficiently [10]. Depending on the molecular weight and the structure, PEIs are divided into low and high molecular weight PEIs and linear and branched PEIs [7]. The transfection efficiency and cytotoxicity of PEI-derived gene vectors strongly depend on the molecular weight [8,11], and the high molecular weight PEI has higher transfection activity and higher cytotoxicity [11]. For example, the well-known commercial linear or branched 25 kDa PEI, as an effective transfection reagent in vitro and in vivo, has high transfection efficiency but has high cytotoxicity at the same time [4]. The low molecular weight PEI (<2 kDa) has much less toxicity but almost no transfection activity [12]. Especially, PEI (800 Da) exhibits negligible cytotoxicity [13]. Considering the advantages of PEIs with different molecular weights, researchers paid much attention to develop potential gene vectors based on PEIs with higher transfection efficiency as well as lower cytotoxicity. For example, the degradable PEI-derived gene vectors were obtained via crosslinking [14–16]. Besides, the biocompatible polymers, such as the cyclodextrin [17], PEG [18], and chitosan [6,19] were also introduced into the PEI based gene vectors to reduce the cytotoxicity.

Recently, dextran, a typical natural biodegradable hydrophilic polysaccharide, containing 1, 6-linked D-glucopyranose residue, has been used for the delivery of plasmid DNA [1,4,20–24]. Dextran is susceptible to enzymatic digestion in human body [25], which would be favorable to decrease the cytotoxicity. When dextran is introduced into polycation vectors, the anhydroglucose units are often broken by oxidization reaction with potassium periodate to obtain the activated

* Corresponding authors. Tel.: +86 27 6875 4061; fax: +86 27 6875 4509.
E-mail addresses: xz-zhang@whu.edu.cn (X.-Z. Zhang), bmp@whu.edu.cn (R.-X. Zhuo).

0168-3659/$ – see front matter © 2008 Published by Elsevier B.V.
doi:10.1016/j.jconrel.2008.03.004

dextran [20–24]. In some reports, the dextran-based polycations were synthesized by conjugation of various oligoamines and spermine to oxidized dextran. But the transfection efficiency of those vectors was generally low except for the dextran–spermine conjugation [21–24]. In contrast to conjugation of various oligoamines to dextran, Tseng et al. conjugated the lower molecular weight dextran to higher molecular weight linear and branched 25 kDa PEIs. The prepared dextran–PEI conjugations had decreased cytotoxicity, high transfection efficiency as well as enhanced stability of complexes in comparing with 25 kDa PEIs [4,20].

In this study, in order to keep the intact structure and biocompatible properties of dextran, dextrans with different molecular weights were functionalized by hexamethylenediisocyanate (HMDI). Then, the branched low molecular weight 800 Da PEI was grafted to the two functionalized dextrans to obtain (Dex-HMDI)-g-PEIs with different molecular weights as gene vectors. The effects of molecular weight of dextran on the physiochemical characteristics, morphology, in vitro cytotoxicity and transfection efficiency of the (Dex-HMDI)-g-PEIs were evaluated.

2. Materials and methods

2.1. Materials

Dextrans with average molecular weights of 15–25 kDa and 60–90 kDa were purchased from Sigma-Aldrich and dried at 60 °C in a vacuum oven for 2 days. Branched polyethylenimines with molecular weight of 25 kDa and 800 Da were purchased from Sigma-Aldrich. Hexamethylenediisocyanate (HMDI, 98%) was purchased from Alfa Aesar. The dimethyl sulphoxide (DMSO) was obtained from Shanghai Chemical Reagent Co., China, which was dried refluxing with anhydrous $MgSO_4$ overnight and was then distilled under reduced pressure. QIAfilter™ plasmid purification Giga Kit (5) was purchased from Qiagen (Hilden, Germany). GelRed™ was purchased from Biotium (CA, USA). Dulbecco's Modified Eagle's Medium (DMEM), fetal bovine serum (FBS), penicillin–streptomycin, trypsin, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), and Dubelcco's phosphate buffered saline (PBS) were purchased from Invitrogen Corp. Micro BCA protein assay kit was purchased from Pierce. All other reagents were analytical grade and used as received.

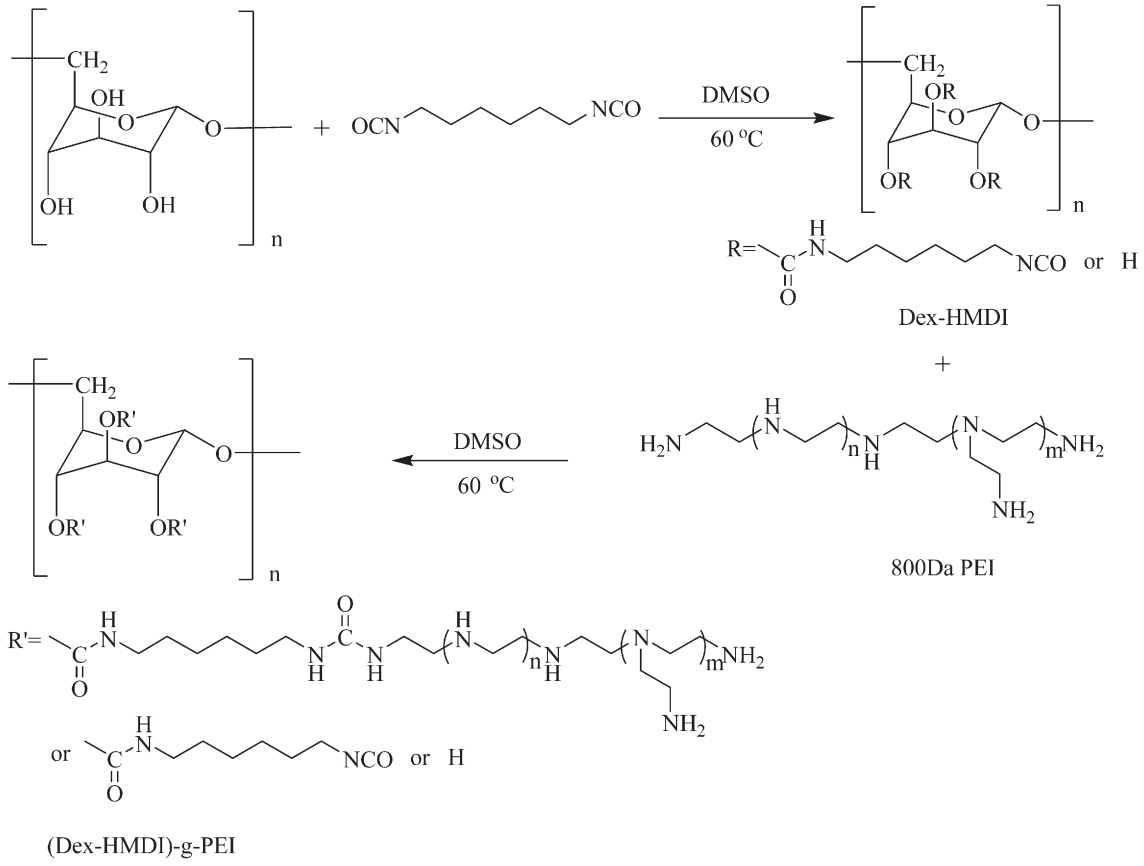
2.2. Synthesis of polymers

2.2.1. Synthesis of functionalized dextrans (Dex-HMDIs)

Two Dex-HMDIs were synthesized according to a literature procedure [26–28], using dextrans with average molecular weights of 15–25 kDa and 60–90 kDa, respectively. In brief, dextran (0.162 g) was dissolved in 10 mL of DMSO at 40 °C. The 15-fold excess HMDI (7.6 g, 7.3 mL) was dissolved in 10 mL of DMSO at 60 °C under nitrogen atmosphere in a 50 mL-flask fitted with a condenser. The dextran solution was added dropwise to the HMDI solution under stirring at 60 °C under nitrogen atmosphere. After 4 h, the reaction solution was precipitated with an excess of diethyl ether to remove residual HMDI. The precipitation was obtained by centrifugal effect and washed 3 times with diethyl ether. Then an ice-cream waxy product was obtained and defined as Dex-HMDI.

2.2.2. Synthesis of (Dex-HMDI)-g-PEIs

Two (Dex-HMDI)-g-PEIs, (Dex_{15k}-HMDI)-g-PEI and (Dex_{60k}-HMDI)-g-PEI, were synthesized by grafting PEI (800 Da) to dextrans with average molecular weights of 15–25 kDa and 60–90 kDa, respectively. For each grafting reaction, 0.6 g PEI was added to 10 mL of DMSO at 60 °C under nitrogen atmosphere in a 50 mL-flask fitted with a condenser. Dex-HMDI was dissolved in 10 mL of DMSO and then the



Scheme 1. Synthesis illustration of (Dex-HMDI)-g-PEI.