Acta Biomaterialia 12 (2015) 156–165

Contents lists available at ScienceDirect

Acta Biomaterialia

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

A general strategy to prepare different types of polysaccharide-*graft*-poly(aspartic acid) as degradable gene carriers

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ARTICLE INFO

Article history:
Received 8 June 2014
Received in revised form 3 October 2014
Accepted 29 October 2014
Available online 4 November 2014

Keywords:
Poly(aspartic acid)
β-Cyclodextrin
Dextran
Chitosan
Gene vectors

ABSTRACT

Owing to their unique properties such as low cytotoxicity and excellent biocompatibility, poly(aspartic acid) (PAsp) and polysaccharides are good candidates for the development of new biomaterials. In order to construct better gene delivery systems by combining polysaccharides with PAsp, in this work, a general strategy is described for preparing series of polysaccharide-*graft*-PAsp (including cyclodextrin (CD), dextran (Dex) and chitosan (CS)) gene vectors. Such different polysaccharide-based vectors are compared systematically through a series of experiments including degradability, pDNA condensation capability, cytotoxicity and gene transfection ability. They possess good degradability, which would benefit the release of pDNA from the complexes. They exhibit significantly lower cytotoxicity than the control 'gold-standard' polyethylenimine (PEI, ~25 kDa). More importantly, the gene transfection efficiency of Dex- and CS-based vectors is 12–14-fold higher than CD-based ones. This present study indicates that properly grafting degradable PAsp from polysaccharide backbones is an effective means of producing a new class of degradable biomaterials.

1. Introduction

Gene therapy is a method showing future promise for conquering intractable diseases [1–4]. The challenging task at present is to find good gene delivery vectors with low cytotoxicity, high transfection efficiency and targeting ability [5–8]. Gene vectors currently include both viral and nonviral vector systems [9]. Compared with viral vectors, synthetic nonviral vectors exhibit low host immunogenicity and can be produced in large quantities [10–13]. Until now, many types of nonviral polycations have been reported to deliver genes, e.g. polyethylenimine (PEI) [14], poly(L-lysine) [15,16], polyamidoamine [17], chitosan (CS) [18–20] and polysaccharide-based carriers [21].

Polysaccharides play a major role in the preparation of new gene vectors [22]. Natural polysaccharides are renewable materials with low toxicity and excellent biocompatibility. Cyclodextrins (CD) composed of α(1–4)-linked glucose units have the advantage of membrane permeation in addition to the common virtues of polysaccharides [23]. Many CD-based gene vectors have been reported already, including CD-embedding polymers [24] and CD-based polyrotaxanes [25]. Dextran (Dex), a branched polysaccharide composed of α(1–6)-linked glucose units, is used extensively to develop new gene vectors [22,26–28]. CS, a family of linear binary polysaccharides derived from chitin [29,30], have been extensively studied as nonviral gene delivery vectors with the special advantage of positive charge [31]. In contrast to low-molecular-weight CS, the high-molecular-weight ones can be complexed well, preferably with DNA, but their water solubility is poor at physiological pH [32,33].

Recently, polypeptide-based gene vectors have also attracted more attention. Cell membrane consists of many types of polypeptides. It is ideal for chemical structure of cationic gene vectors to mimic polypeptides [34]. Polyamino acid with good degradability and structures similar to polypeptides is the best candidate for the development of new effective gene vectors. Different types of aminolyzed poly(aspartic acid) (PAsp) prepared by the ring-opening reaction of *β*-benzyl-L-aspartate *N*-carboxy anhydride (BLA-NCA) have been reported as gene vectors with high transfection and low cytotoxicity [35–39]. More recently, the present authors reported different types of linear and star-shaped low-molecular-weight poly(2-(dimethylamino)ethyl methacrylate)-functionalized PAsp for efficient gene delivery. The star-shaped PAsp contained CD cores and exhibited better gene delivery performance [10].

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http://dx.doi.org/10.1016/j.actbio.2014.10.041
1742-7061/

Further improvement in delivery performance of the PAsp-based vectors is still needed to facilitate their application.

Different polysaccharides possess different physiochemical properties that would affect the resultant gene delivery capabilities. In order to construct better gene delivery systems by combining polysaccharides with PAsp, it is necessary to develop a simple strategy to functionalize different types of polysaccharides with PAsp. The present work reports a general strategy for preparing different types of polysaccharide-*graft*-PBLA polymers (including CD, Dex and CS) via ring-opening polymerization of BLA-NCA, which were initiated by primary amine groups of CS and ethylenediamine (ED)-functionalized CD or Dex (Fig. 1). Polysaccharide-*graft*-PBLA was subsequently aminolyzed with ED to produce new degradable gene vectors. The different types of polysaccharide-based carriers were investigated and compared systematically through a series of experiments, including degradability, pDNA condensation capability, cytotoxicity and gene transfection ability. The present study provides valuable information for constructing new polysaccharide-based degradable delivery systems.

2. Materials and methods

2.1. Materials

Branched PEI (M_w ~25,000 Da), β-cyclodextrin (β-CD, >98%), 1,1′-carbonyldiimidazole (CDI, 97%), dextran (Dex, M_r ~6000), triphosgene (>99%), anhydrous dimethyl sulfoxide (DMSO) and ED (>98%) were purchased from Sigma–Aldrich Chemical Co., St. Louis, MO. Anhydrous BLA > 98%) was purchased from Tokyo Chemical Industry Co. Ltd, Japan. CS (degree of deacetylation ~55%, M_n ~6100; Table 1) was obtained from Jinan Haidebei Ltd, China. The monomer BLA-NCA was prepared using the Fuchs–Farthing method and the detailed synthesis process was described in earlier work [10]. Tetrahydrofuran, acetic ether and n-hexane were dried over CaH_2 for about a week and distilled over fresh CaH_2 powder under normal pressure. Other solvents were used directly without any treatment. COS7 and HepG2 cell lines were purchased from the American Type Culture Collection (ATCC, Rockville, MD). The cells with passage number 3 were used for gene transfection assay.

Table 1
Characterization of the polymers.

Samples	M_n (g mol^{-1})[d]	PDI[d]	Monomer repeat units per side chain[e]
CD-NH2[a]	1.69×10^3	1.07	
Dex-NH2[b]	7.93×10^3	1.43	
CS	6.14×10^3	1.24	
CD-PAsp-ED-1[c]	4.92×10^3	1.49	5.1
CD-PAsp-ED-2[c]	9.17×10^3	1.29	11.9
CD-PAsp-ED-3[c]	1.44×10^4	1.17	20.2
Dex-PAsp-ED-1[c]	2.05×10^4	1.18	4.7
Dex-PAsp-ED-2[c]	3.90×10^4	1.45	10.6
Dex-PAsp-ED-3[c]	6.22×10^4	1.51	19.5
CS-PAsp-ED-1[c]	2.16×10^4	1.26	5.5
CS-PAsp-ED-2[c]	4.02×10^4	1.25	12.1
CS-PAsp-ED-3[c]	6.37×10^4	1.34	22.3

[a] Synthesized using a molar feed ratio [CD]:[CDI] of 1:8 at room temperature for 24 h, followed by adding excessive ED for another 48 h.
[b] Synthesized using a molar feed ratio [Dex]:[CDI] of 1:30 at room temperature for 24 h, followed by adding excessive ED for another 48 h.
[c] Synthesized using different molar feed ratio [BLA-NCA]:[CD-NH_2 or Dex-NH_2 or CS] at 50 °C in 8 ml of anhydrous DMSO for 72 h, followed by aminolysis, where the [amino group]:[BLA-NCA] molar feed ratios were 1/30, 1/50, and 1/100, respectively.
[d] Determined from GPC results. PDI = weight average molecular weight/number average molecular weight, or M_w/M_n.
[e] Determined from M_n of CD-PAsp-ED or Dex-PAsp-ED or CS-PAsp-ED and M_n of CD-NH_2 or Dex-NH_2 or CS.

2.2. Preparation of CD-NH_2 and Dex-NH_2

The hydroxyl groups of β-CD (or Dex) were first activated by CDI, and a solution of CDI (7.04 mmol in 4 ml of anhydrous DMSO)

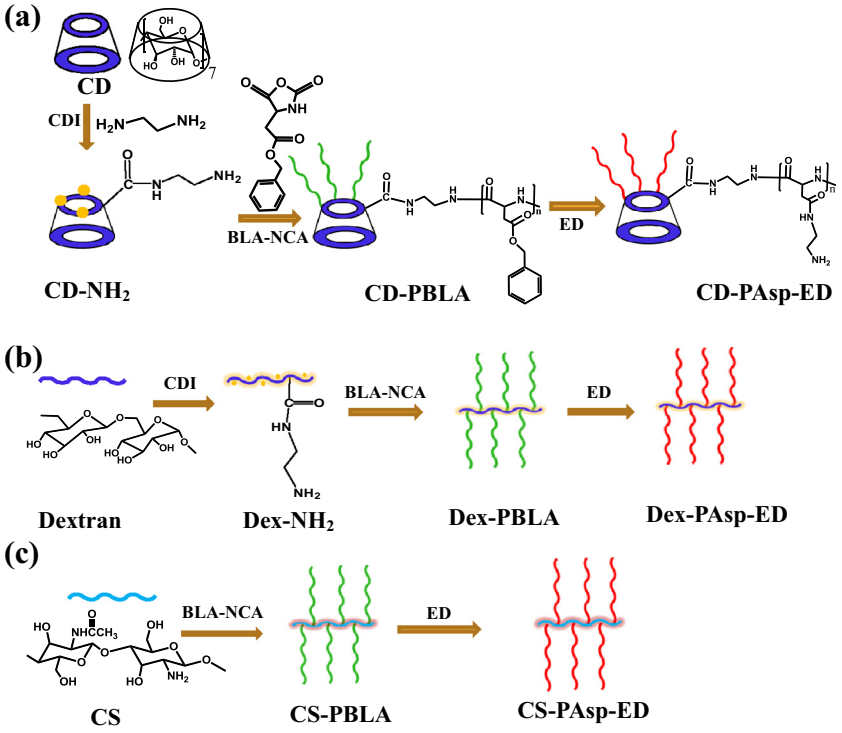

Fig. 1. Schematic diagram illustrating the preparation processes of degradable CD-PAsp-ED, Dex-PAsp-ED and CS-PAsp-ED.