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Different types of degradable vectors from low-molecular-weight polycation-functionalized poly(aspartic acid) for efficient gene delivery

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ABSTRACT

Poly(aspartic acid) (PAsp) has been employed as the potential backbone for the preparation of efficient gene carriers, due to its low cytotoxicity, good biodegradability and excellent biocompatibility. In this work, the degradable linear or star-shaped PBLA was first prepared via ring-opining polymerization of β-benzyl-L-aspartate *N*-carboxy anhydride (BLA-NCA) initiated by ethylenediamine (ED) or ED-functionalized cyclodextrin cores. Then, PBLA was functionalized via aminolysis reaction with low-molecular-weight poly(2-(dimethylamino)ethyl methacrylate) with one terminal primary amine group (PDMAEMA-NH_2), followed by addition of excess ED or ethanolamine (EA) to complete the aminolysis process. The obtained different types of cationic PAsp-based vectors including linear or star PAsp-PDM-NH_2 and PAsp-PDM-OH exhibited good condensation capability and degradability, benefiting gene delivery process. In comparison with gold standard polyethylenimine (PEI, ~25 kDa), the cationic PAsp-based vectors, particularly star-shaped ones, exhibited much better transfection performances.

1. Introduction

Gene therapy has attracted intense research interest as an effective way to cure cancer and genetic disorders. However, the design of gene delivery vectors with low cytotoxicity and high transfection efficiency remains a great challenge [1,2]. Compared with viral vectors, cationic polymers as the major type of nonviral gene delivery vectors exhibit advantages of low host immunogenicity and large–scale production. A large number of polycations, including polyethylenimine (PEI) [3,4], poly(2-(dimethylamino) ethyl methacrylate) (PDMAEMA) [4], poly(L-lysine) [5], Poly(-aspartic acid) [6,7], polyamidoamine [8], and cyclodextrin (CD)-based cationic carriers [9–11], have been reported to deliver nucleic acids. Polypeptides had been widely used in biomedical field since they are non-toxic, biocompatible, and biodegradable. In particular, poly(aspartic acid) (PAsp)-based nanoparticles [12,13], micelles [14–18], nanocapsules [19] and membranes [20] were fabricated for the delivery of drug, DNA, RNA and proteins. The application of PAsp in gene carriers has not been investigated widely and thoroughly. Further improvement in PAsp-based vectors will benefit constructing better gene-delivery systems.

PDMAEMA is a common polycation for gene carrier, while its high transfection efficiency is generally associated with a devastating toxicity [4]. Low-molecular-weight polycations exhibit low cytotoxicity but low transfection efficiency. It was reported that in comparison with high-molecular-weight cationic homopolymers, the comb cationic copolymer composed of one biocompatible backbone and low-molecular-weight polycation side chains exhibited much higher gene transfection efficiency and lower cytotoxicity [21,22]. Star-shaped cationic polymers have recently attracted considerable attention as gene carriers because of their dense molecular architecture with moderate flexibility [23]. CDs are a series of cyclic oligosaccharides composed of D(+)-glucose units linked by α-1,4-linkage, which could improve gene bioavailability via enhancing cell membrane absorption or stabilizing gene in physiological media [21,24]. A series of CD-cored star-shaped cationic polymers have been designed for the efficient delivery of nucleic acids [25–29]. However, CDs had not been used to produce star-like PAsp-based gene vectors.

In the present work, a series of linear or star-shaped PAsp-based gene vectors were prepared via ring-opining polymerization of β-benzyl-L-aspartate *N*-carboxy anhydride (BLA-NCA) initiated by ethylenediamine (ED) or ED-functionalized CD cores, followed by functionalization via aminolysis reaction with low-molecular-weight PDMAEMA (Fig. 1). Such different types of PAsp-based

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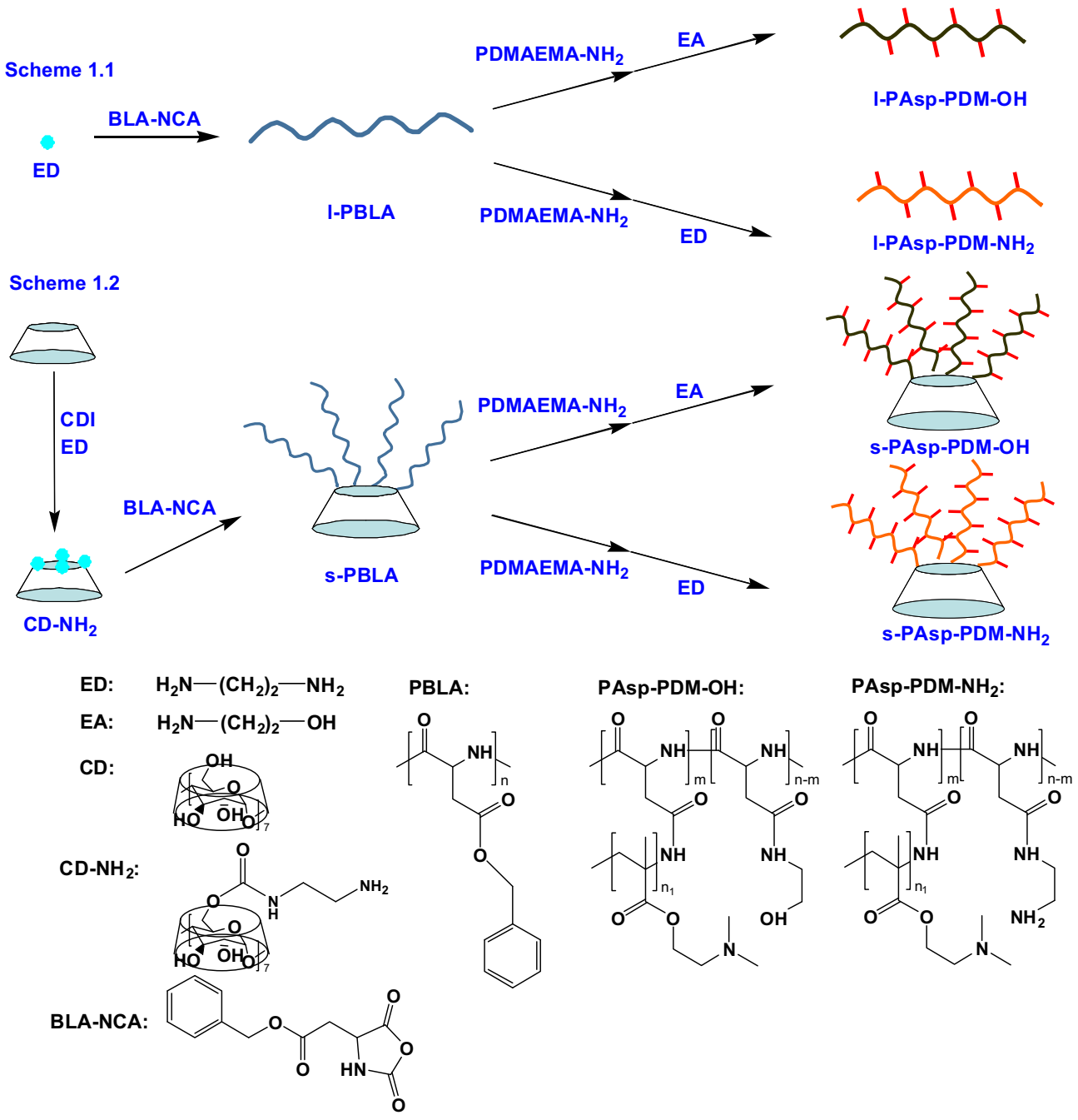

Fig. 1. Schematic diagram illustrating the preparation processes of degradable l-PAsp-PDMAEMA and s-PAsp-PDMAEMA.

carriers were investigated in detail through a series of experiments including degradability, DNA binding capability, cytotoxicity, and gene transfection ability. The current study would provide the useful information for constructing better PAsp-based delivery systems with good biophysical properties.

2. Materials and methods

2.1. Materials

Branched polyethylenimine (PEI, M_w ~25,000 Da), 1,1′-carbonyldiimidazole (CDI, 97%), triphosgene (>99%), 2-(dimethylamino)ethyl methacrylate (DMAEMA, >98%), copper(I) bromide (CuBr, 99%), *N,N,N′,N″,N″*-pentamethyldiethylenetriamine (PMDTETA, >98%), anhydrous dichloromethane (ultra drying, $H_2O \leq 0.004\%$), ethanolamine (EA, >98%), ethyl 2-bromoisobutyrate (>98%), calcium hydride (CaH_2, AR), fluorescein diacetate (FDA, >98%), propidium iodinate (PI, >98%), D-mannitol (>99%) 3-(4,5-dimethylthiazol-2yl)-2,5-diphenyl tetrazolium bromide (MTT), penicillin, and streptomycin were obtained from Sigma–Aldrich Chemical Co., St. Louis, MO. DMAEMA was used after removal of the inhibitors in a ready-to-use disposable inhibitor-removal column (Sigma–Aldrich). Anhydrous 1,4-dioxane L-aspartic acid β-benzyl ester (BLA, >98%), anhydrous *N,N*-dimethylformamide (DMF), and ethylenediamine (ED, >98%) were purchased from Tokyo Chemical Industry Co. Ltd, Japan. Acetic ether and n-hexane were dried over CaH_2 for at least a week and were distilled over fresh CaH_2 powder under a normal pressure. Other solvents were directly used without treatment. HEK293 and HepG2 cell lines were purchased from the American Type Culture Collection (ATCC, Rockville, MD).

2.2. Synthesis of monomer

β-Benzyl-L-aspartate N-carboxy anhydride (BLA-NCA) was prepared using the Fuchs-Farthing method [30,31]. BLA (6 g) was reacted with triphosgene (3 g, about 1.4 equiv) in 50 mL of anhydrous 1,4-dioxane under a nitrogen atmosphere at 50 °C until the solution became clear. Anhydrous n-hexane was slowly added to the