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Research paper

An efficient vector for gene delivery: α,β-poly (3-dimethylaminopropyl-D,L-aspartamide)

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Abstract

PSI, as the potential peptide-like intermediate, is subject to simple chemical modification in order to obtain good non-viral carriers for gene delivery. This paper describes the facile synthesis and preliminary evaluation of α,β-poly (3-dimethylaminopropyl-D,L-aspartamide) (PDAI) as a vector. Reaction of PSI with 3-dimethylamino-1-propylamine afforded PDAI in *N*,*N*-dimethylformamide (DMF) solution. Such biophysical properties of PDAI/DNA complexes as the particle size and the zeta potential were determined by dynamic light scattering assay. The complexes prepared at weight ratios ranging from 2 to 3 have an average size of around 200 nm and a zeta potential of around 10.0 mV. Gel electrophoresis assays confirmed that PDAI could compact DNA to form the complexes and protect DNA from enzymatic degradation by DNase I at the weight ratio above 2.0. Furthermore, PDAI was found to transfect HepG2 cells at a much higher efficiency than commercially available polyethylenimine (PEI) ($W_w = 75{,}000$ Da). MTT cytotoxicity assay demonstrated that PDAI also showed much less toxicity than did PEI, suggesting that PDAI is a new class of transfection reagent to be used as a safe vector.

Keywords: PDAI; PDAI/DNA complex; Gene delivery; Transfection efficiency

1. Introduction

Gene therapy holds a prospect for treatment and prevention of congenital and acquired diseases by introducing small DNA or RNA sequences into cells. The type of vector used is a key to success in gene delivery [1]. So far there have been two main approaches to gene delivery concerning both viral and non-viral vectors. Besides viral gene carriers, non-viral gene delivery systems such as cationic polymers are being investigated intensively to circumvent some of the problems encountered in using viral vectors [2,3]. To enhance transfection efficacy numerous cationic polymers [4,5] have been used for transfection. Though these polycations improve transfection in comparison to naked DNA, they still have relatively low transfection efficacy, or they are associated with the toxicity in vitro or in vivo, or their preparation is complicated.

Recently biodegradable polycations with hydrolysable chemical bonds are now emerging as a new generation of synthetic carriers. Peptide derivatives of poly-D,L-aspartamide (PSI) are gaining much attention. They show both flexibility and advantage in designing drug carriers [6–14] such as a large amount of drug loading capacity and the potential for low toxicity and superior biocompatibility. However, polycations[13,14] based on PSI are comparatively rare, their synthesis is also inconvenient.

We herein report our effort toward designing a new derivative of PSI, α,β-poly (3-dimethylaminopropyl-D,L-aspartamide) (PDAI), as a potential carrier for gene delivery. PDAI

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vector was conveniently synthesized from ring-opening reactions of primary amine to PSI. The polymer was characterized by NMR, elemental analysis and gel permeation chromatography (GPC). Its DNA complexation, ability to protect DNA against enzymatic degradation, and cytotoxicity were investigated. The transfection efficiency of PDAI/DNA complexes was evaluated in HepG2 cells using the reporter gene β-galactosidase. Lipofectin® and polyethylenimine were used as positive controls.

2. Materials and methods

2.1. Materials

D,L-Aspartic acid, linear poly (ethylenimine) (PEI) ($M_w = 75{,}000$ Da), *O*-nitrophenyl-β-D-galactopyranoside (ONPG), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) were all purchased from Sigma Chemical Company (St. Louis, MO). Lipofectin® and RPMI Medium 1640 were obtained from Gibco, BRL (Grand Island, NY). The pSV-galactosidase control vector with SV40 early promoter, enhancer and *LacZ* gene was supplied by Promega Corporation (Madison, WI). The pSV-galactosidase plasmid was amplified in *Escherichia coli*, and then the plasmids were isolated and purified.

2.2. Preparation and characterization of PDAI

Poly-D,L-succinimide (PSI) was prepared as described in the literature [6]. Briefly, D,L-aspartic acid (10 g) was mixed with 5 g of 85% phosphoric acid in the flask. The flask was heated in an oil bath at 180 °C for 4 h. The vitreous mass obtained was dissolved into 50 ml of *N*,*N*-dimethylformamide (DMF) and the solution was poured into a beaker containing 200 ml of water. A flaky precipitation formed which was centrifuged, washed with water until neutral, and dried.

A solution of 3 g PSI in 15 ml DMF was separated and added to 10 ml *N*,*N*-dimethylamino-1-propylamine (DAP) or ethanolamine. The reaction mixture was rapidly stirred at ambient temperature for 24 h. The solution was then acidified with glacial acetic acid to pH 4. To remove excess DAP or ethanolamine, the solution was diluted with water, dialyzed against deionized water and lyophilized.

The NMR spectra of PDAI and PHEA were recorded with a Bruker DRX-500 NMR spectrometer in D_2O solution. Infrared spectra were recorded with a Nicolet 170SX FT-IR spectrometer. Samples were pressed into KBr pellets. The elemental compositions of PDAI and PHEA were determined by elemental analysis (EA-240C, Perkin-Elmer). The average molecular weight (M_w) and polydispersity (PD) of PDAI and PHEA were determined by GPC (TL-9800, Shimadzu) with a TSK-gel column (G3000SW, Tosoh Corporation, Japan). GPC was performed at a flow rate of 0.5 ml/min, the mobile phase was 1% acetic acid. The calibration curve was prepared with protein standards of different molecular weight including rabbit phosphorylase B (97,400 Da), bovine serum albumin (66,200 Da), rabbit actin (43,000 Da), bovine carbonic anhydrase (31,000 Da), trypsin inhibitor (20,100 Da) and hen egg white lysozyme (14,400 Da).

2.3. Preparation and characterization of PDAI/DNA complexes

PDAI was dissolved in 0.5% acetic acid. The pH of the solutions was adjusted to 5.0 with NaOH. The final concentration was 1 mg/ml. Then the solution was sterile filtered through a 0.22 μm filter, and diluted to 0.5 mg/ml by RPMI 1640.

The required volume of 500 μg/ml PDAI solution was added to PBS containing 50 μl of 0.2 mg/ml DNA (diluted from 1 mg/ml by RPMI 1640 by gentle pipetting to form complexes of a selected weight ratio). The mixture was vortexed rapidly for several seconds and left for 30 min to ensure the complexes would completely form at room temperature.

The diameters and zeta potentials of the complexes were determined by photon correlation spectroscopy by using a 90 Plus Particle Sizer (Brookhaven Instruments, Holtsville, NY). The physical stability of each complex was studied by agarose gel electrophoresis (0.9% agarose in TAE buffer).

2.4. Nuclease resistance of PDAI/DNA complexes

Each sample contained 2 μg DNA complexed with PDAI at different weight ratios. After incubation for 30 min at room temperature, 200 U/ml DNase I (5 units) buffer (50 mM Tris–Cl, pH 7.6, and 10 mM $MgCl_2$) was added, and each sample was incubated at 37 °C. Samples were then loaded onto a 0.8% agarose gel stained with EtBr and subjected to electrophoresis.

2.5. Cytotoxicity evaluation

Cytotoxicity of different reagents was measured using the MTT dye reduction assay. HeLa, L929 and HepG2 cells were seeded in a 96-well plate at a density of 2.0×10^4 cells/well and incubated overnight as described in the transfection session. Then the cells were incubated in 100 μl serum free medium containing selected amount (from 1 to 35 μg) of PDAI, PHEA and PEI. After 12 h, the medium was removed and the cells were rinsed twice with PBS. The wells were refilled with complete medium and cells were cultured for another 24 h. Next, 20 μl of MTT (5 mg/ml) solution was added into each well and was allowed to react for 4 h at 37 °C. A total of 200 μl of DMSO was added to each well and the plate was incubated for 30 min at room temperature. Absorbance at 490 nm was measured with an ELISA plate reader (Bio-Rad, Microplate Reader 3550).