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Comparison of ethanolamine/ethylenediamine-functionalized poly(glycidyl methacrylate) for efficient gene delivery

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ABSTRACT

Cationic polymers with low cytotoxicity and high transfection efficiency have attracted considerable attention as non-viral carriers for gene delivery. Recently we reported that ethanolamine (EA)-functionalized poly(glycidyl methacrylate) (PGMA) (termed PGEA) vectors can have excellent transfection efficiency, while exhibiting very low toxicity. Herein different EA- and ethylenediamine (ED)-functionalized PGMA (termed PGEAED) vectors, as well as ED-functionalized PGMA (termed PGED) vectors, are proposed and compared for efficient gene delivery. In addition to the cationic pendant secondary amine and hydroxyl groups of PGEA, PGEAED, and PGED also contain flanking primary amine groups. PGEAED and PGED exhibited a substantially enhanced ability to condense pDNA into complex nanoparticles at the 100 nm level with positive zeta potentials of about 30 mV at nitrogen/phosphate (N/P) ratios of 10 or higher. More interestingly, no obvious change in the cytotoxicity of PGEAED was observed with a substantial increase in ED content. Moreover, the flanking primary amine groups induced by ED could be readily functionalized by glycyrrhetinic acid or cholic acid to improve the biophysical properties of the gene vectors.

1. Introduction

Successful gene therapy depends on the design of safe and efficient vectors [1–4]. In comparison with viral vectors and cationic lipids, cationic polymers show low host immunogenicity, high flexibility, and easy preparation [5,6]. Over the last few decades cationic polymers have been receiving considerable attention as a major type of non-viral gene delivery nanovectors. A large number of polycations, including polyethylenimine (PEI) [7], poly(tertiary amine methacrylate) [8–10], poly(L-lysine) [11], polyamidoamine [12], and cationic polysaccharides, have been reported to deliver nucleic acids. A high transfection efficiency is generally associated with devastating toxicity. Most of the existing cationic polymers suffer from either a low transfection efficiency or significant toxicity. The epoxide groups of poly(glycidyl methacrylate) (PGMA) can react readily and irreversibly with nucleophilic groups, such as $-NH_2$. It was reported that such aminated PGMA could be used as a safe gene vector [2,13]. We recently reported that ethanolamine (EA)-functionalized PGMA (termed PGEA) vectors can produce excellent transfection efficiency in different cell lines, while exhibiting very low toxicity [14]. In comparison with traditional branched PEI (25 kDa), such PGEA vectors possess plentiful non-ionic hydrophilic hydroxyl units, as well as flanking cationic secondary amine groups.

Ethylenediamine (ED) (M_w = 60 g mol^{-1}) has a similar molecular mass to EA (M_w = 61 g mol^{-1}), but ED possesses double amino groups. These issues motivated us to investigate the structural effects of EA and ED on transfection efficiency, as well as cytocompatibility, when constructing better gene delivery systems. In the present work different EA- and ED-functionalized PGMA (PGEAED) vectors, as well as ED-functionalized PGMA (PGED) vectors are proposed and compared (Fig. 1). In addition to the pendant secondary amine and hydroxyl groups, PGEAED and PGED also contain flanking primary amine groups. The pDNA condensation ability, cytotoxicity, and transfection efficiency of PGEAED and PGED were investigated systematically. The further functionalization of PGED by biomolecules containing carboxyl groups was also examined.

2. Materials and methods

2.1. Ethanolamine/ethylenediamine-functionalized poly(glycidyl methacrylate)

The different functionalized PGMA vectors were prepared by reacting a well-defined PGMA (~90 GMA units, $M_n = 1.28 \times 10^4$ g mol^{-1}, PDI = 1.23) with EA/ED (Fig. 1). Details of the preparation of PGMA have been described earlier [14]. For the functionalized PGMA vectors 1.0 g of PGMA was dissolved

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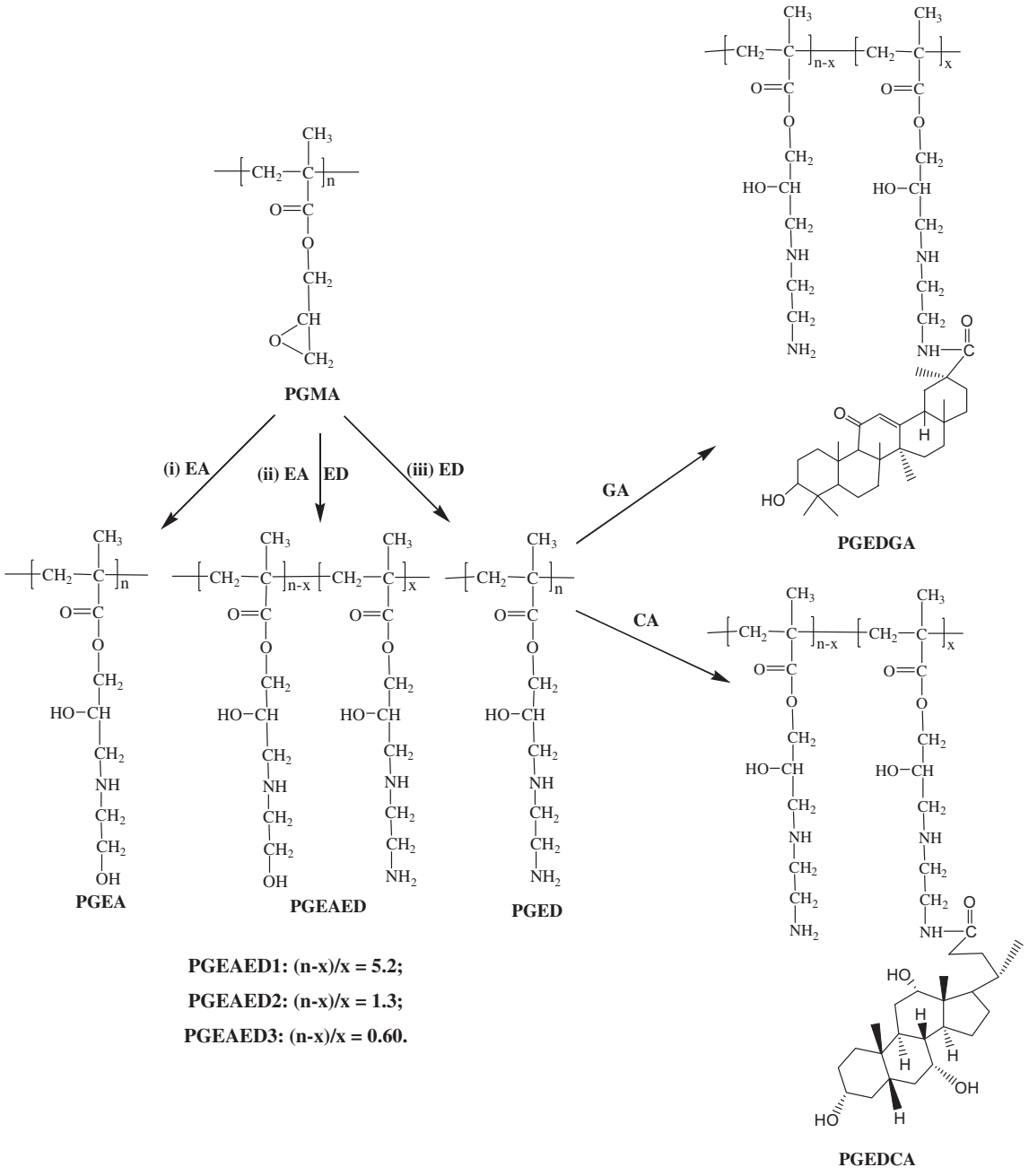

Fig. 1. Preparation of different ethanolamine (EA)/ethylenediamine (ED)–functionalized poly(glycidyl methacrylate) (PGMA) vectors and functionalization of PGED vectors with biomolecules. GA, glycyrrhetinic acid; CA, cholic acid.

in 20 ml of tetrahydrofuran/dimethylformamide mixture (50/50 vol.%). Twenty milliliters of EA/ED (100/0 vol.% for PGEA; 80/20 vol.% for PGEDED1; 60/40 vol.% for PGEAED2; 40/60 vol.% for PGEAED3; 0/100 vol.% for PGED) and 4 ml of triethyleneamine (TEA) was then added. The reaction mixture was stirred at 55 °C for 72 h to produce the functionalized PGMA carriers. The final reaction mixture was precipitated and washed with excess diethyl ether prior to being redissolved in 15 ml of deionized water and dialyzed against deionized water (4 × 5 l) using a 3.5 kDa molecular weight cut-off dialysis membrane at room temperature for 48 h. The final products were freeze dried.

The functionalization of PGED vectors with biomolecules was carried out via the reaction of $-NH_2$ groups of PGED with –COOH groups of glycyrrhetinic acid (GA) or cholic acid(CA), producing the PGEDGA or PGEDCA vectors. GA or CA, 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDAC) and N-hydroxysuccinimide (NHS) at a feed molar ratio of 1:1.2:1.2 were dissolved in 3 ml of dimethyl sulfoxide (DMSO), with the feed molar ratio of GA or CA and $-NH_2$ groups of PGED (0.2 g) maintained between 5% and 20%. The mixture of GA or CA, EDAC, and NHS was stirred at room temperature for 2 h, mixed with 4 ml of DMSO containing 0.2 g of PGED and 0.5 ml of TEA and stirred for another 24 h at room temperature. At the end of the reaction the reaction mixture was precipitated and redissolved in 5 ml of deionized water and dialyzed against deionized water (4 × 5 L) using a 3.5 kDa molecular weight cut-off dialysis membrane at room temperature for 24 h.

2.2. Characterization of polymer/plasmid DNA complexes

The plasmid (encoding *Renilla* luciferase) used in this work was pRL-CMV (Promega Co., Cergy Pontoise, France), which was cloned originally from the marine organism *Renilla reniformis*. The plasmid