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Aminated Poly(glycidyl methacrylate)s for Constructing Efficient Gene Carriers

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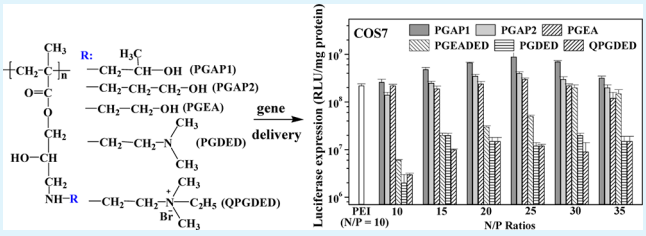
Supporting Information

ABSTRACT: Aminated poly(glycidyl methacrylate) (PGMA) vectors could efficiently mediate gene delivery. Recently, we reported that ethanolamine (EA)-functionalized PGMA could provide high transfection efficiency, while exhibiting very low toxicity. Herein, different amine species, including 1-amino-2-propanol (AP1), 3-amino-2-propanol (AP2), EA, and *N,N,*-dimethylethylenediamine (DED), and its quaternized DED, were proposed to aminate PGMA. The DNA condensation abilities, pH buffering capacities, cytotoxicities, and gene transfection efficiencies of the resultant aminated PGMA vectors were systematically compared. Compared with EA, AP1 (or AP2) contains an additional methyl (or methylene) group. EA-, AP1-, and AP2-functionalized PGMA vectors exhibited similar condensation abilities. The methyl (from AP1) and methylene (from AP2) species could benefit the gene delivery. The transfection performance mediated by AP1-functionalized PGMA is best. DED possesses a tertiary amine group, which could be quaternized to further enhance the DNA condensation ability of aminated PGMA. No obvious increase in cytotoxicity of quaternized DED-aminated PGMA was observed. But both DED- and its quaternized counterpart-functionalized PGMA vectors exhibited very low pH buffering capacities, making them exhibit poor gene transfection performances. The current study would provide useful information for constructing better PGMA-based delivery systems with good biophysical properties.



KEYWORDS: *ethanolamine, 1-amino-2-propanol, 3-amino-2-propanol, N,N,-dimethylethylenediamine, GMA, gene delivery*

INTRODUCTION

The most challenging task in gene therapy is the design of vectors with low cytotoxicity and high transfection efficiency.[1] Compared with viral vectors and cationic lipids, cationic polymers as the major type of nonviral gene vectors have been receiving considerable attention, due to their low host immunogenicity, high flexibility, and easy preparation.[2,3] Over the last few decades, a large number of polycations, including polyethylenimine (PEI),[4] poly(tertiary amine methacrylate),[5,6] dendrimer polycations,[7,8] and cationic polysaccharides,[9,10] have been reported to deliver nucleic acids. Branched PEI (25 kDa) is widely used for nonviral gene delivery due to its high transfection efficiency.[4,9] Under physiological conditions, polycations can spontaneously condense negatively charged DNA into compact nanocomplexes, reduce the electrostatic repulsion between DNA and cell surfaces, protect plasmid DNA (pDNA) from enzymatic degradation by nucleases, and facilitate cellular transfection. However, most of the reported polycations still suffered from either low transfection efficiency or significant toxicity.

Poly(glycidyl methacrylate) (PGMA) can react readily and irreversibly with nucleophilic groups, such as $-NH_2$. It was reported that aminated PGMA could be used as a safe gene vector.[3,11] Such a vector possesses flanking cationic amine species and nonionic hydroxyl units. The cationic amine species are responsible for condensing DNA. Recently, we reported that ethanolamine (EA) or ethylenediamine (ED)-functionalized PGMA (denoted as PGEA[12] or PGED[13]) could efficiently mediate gene delivery in different cell lines, while exhibiting very low toxicity. In comparison with traditional branched PEI (25 kDa), PGEA possesses plentiful nonionic hydrophilic hydroxyl units, as well as flanking cationic secondary amine groups. In addition to the secondary amine and hydroxyl groups, PGED also contains the flanking primary amine groups, which could be readily functionalized by carboxyl group-containing biomolecules to further improve the biophysical properties of PGED. The earlier studies also indicated that the chain lengths of aminated PGMA vectors could significantly affect transfection efficiency.[12,14,15]

In addition to EA and ED, other amine species, including 1-amino-2-propanol (AP1), 3-amino-2-propanol (AP2), and *N,N,*-dimethylethylenediamine (DED), possess similar molecular structures. In comparison with EA, AP1 (or AP2) contains an additional methyl (or methylene) group. In addition to the primary amine group, DED also possesses a tertiary amine group, which could be quaternized to further enhance the DNA condensation ability of gene vectors. These issues motivated us to investigate the structural effects of different types of

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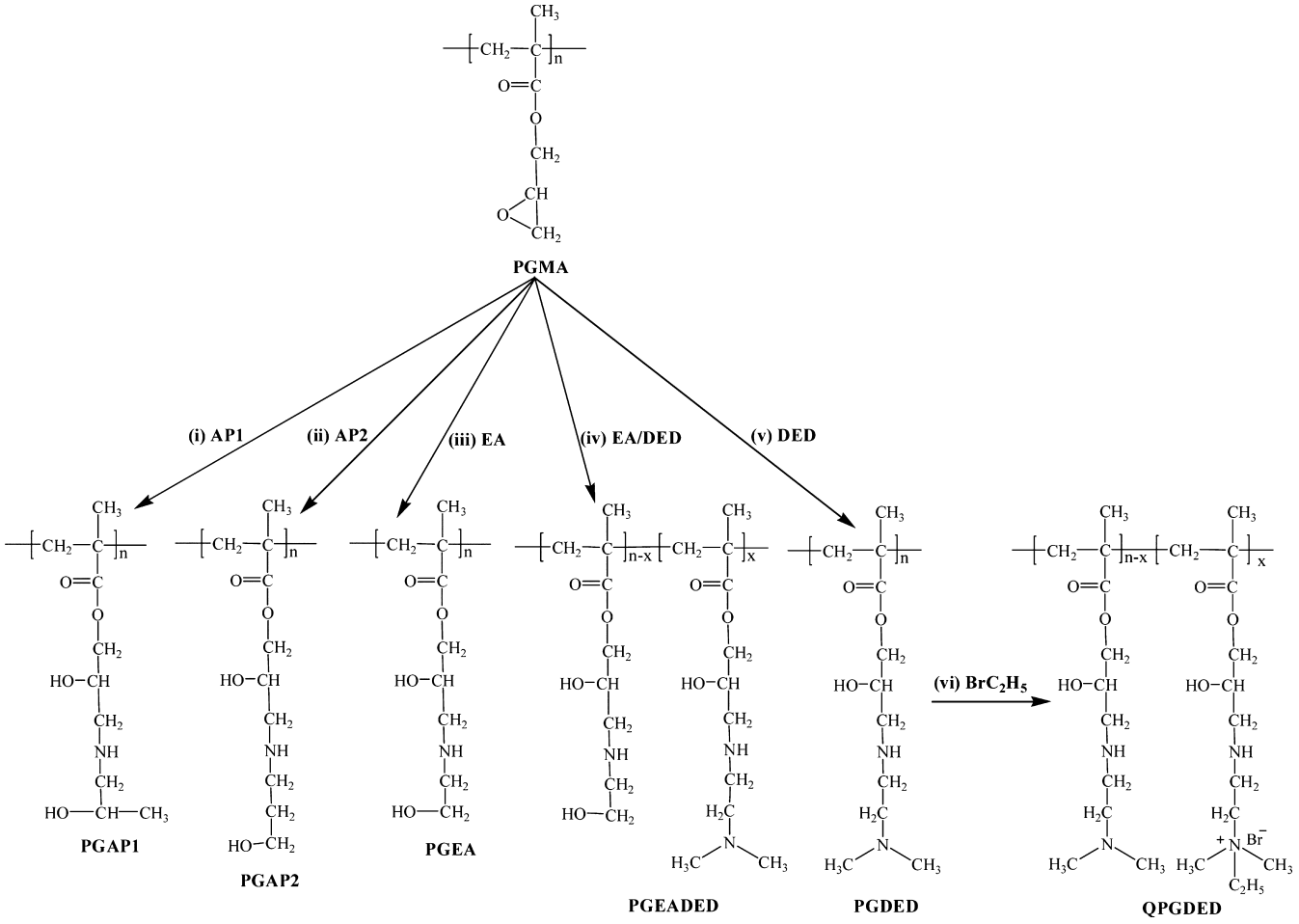

Figure 1. Preparation processes of different amine species-functionalized poly(glycidyl methacrylate) (PGMA) vectors.

aminated PGMA vectors on gene transfection. In the present work, different amine species (including AP1, AP2, EA, EA/DED, DED, and quaternized DED) aminated PGMA vectors were proposed (Figure 1). The systematical comparisons of their DNA condensation abilities, pH buffering capacities, cytotoxicities, and gene transfection efficiencies were performed for constructing better gene-delivery systems.

EXPERIMENTAL SECTION

Materials. Branched polyethylenimine (PEI, M_w ~ 25 000 Da), glycidyl methacrylate (GMA, 98%), ethanolamine (EA, 98%), 1-amino-2-propanol (AP1, 98%), 3-amino-2-propanol (AP2, 98%), *N*,*N*-dimethylethylenediamine (DED, 99%), bromoethane (98%), triethyleneamine (99%), 1,1,4,7,10,10-hexamethyltriethyenetetramine (HMTETA, 99%), ethyl bromoisobutyrate (99%), and copper(I) bromide (CuBr, 99%) were obtained from Sigma-Aldrich Chemical Co., St. Louis, MO. GMA was used after removal of the inhibitors in a ready-to-use disposable inhibitor-removal column (Sigma-Aldrich). 3-(4,5-Dimethylthiazol-2yl)-2,5-diphenyl tetrazolium bromide (MTT), penicillin, and streptomycin were also purchased from Sigma-Aldrich Chemical Co., St. Louis, MO. HEK293 cell lines were purchased from the American Type Culture Collection (ATCC, Rockville, MD).

Different Aminated PGMA Vectors. PGMA was synthesized using a molar feed ratio [GMA (12 mL)]:[ethyl bromoisobutyrate]:[CuBr]:[HMTETA] of 140:1:1:1.5 at 50 °C in 24 mL of GMA/THF (1/1, v/v). The reaction was performed in a 50 mL flask equipped with a magnetic stirrer and under the typical conditions of ATRP. GMA, THF, ethyl bromoisobutyrate, and HMTETA were introduced into the flask. The reaction mixture was degassed by bubbling argon for 20 min. Then, CuBr was added into the mixture, and the flask was then sealed with a rubber stopper under an argon atmosphere. The polymerization was allowed under continuous stirring at 50 °C to produce PGMA from 3 h of ATRP (yield 7.1 g, about 105 GMA units, M_n = 1.52 × 10^4 g/mol, PDI = 1.3). The reaction was stopped by exposing to air. PGMA was precipitated in excess methanol to remove the catalyst complex. The crude polymer was washed by water and purified by reprecipitation cycles to further remove the reactant residues, prior to being dried under reduced pressure. After purification, no signals associated with Cu elements were observed in X-ray photoelectron spectroscopy, indicating that the copper-based catalyst complex had been removed completely from PGMA.

For preparation of aminated PGMA vectors, 0.5 g of PGMA was dissolved in 14 mL of THF/DMF mixture (1/1, v/v). A 2 mL portion of triethyleneamine and 5 mL of AP1 for PGAP1, AP2 for PGAP2, EA for PGEA, EA/DED (mol/mol, 2/1) for PGEADED, or DED for PGDED were then added. The reaction mixture was stirred at 50 °C for 5 days 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) with dialysis membrane (MWCO, 3500 Da) at room temperature for 24 h. The final products were freeze-dried. QPGDED with partially quaternized DED was prepared at 60 °C for 24 h in 5 mL of DMF containing 0.2 g of PGDED and 150 μL of bromoethane. QPGDED was precipitated in excess diethyl ether and dried under reduced pressure.

Characterization of Polymer. The molecular weights of polymers were determined by gel permeation chromatography (GPC) and chemical structures by nuclear magnetic resonance (NMR). GPC measurements of PGMA were performed on a Waters GPC system equipped with Waters Styragel columns, a Waters-2487 dual wavelength (λ) UV detector, and a Waters-2414 refractive index

detector. THF was used as the eluent at a flow rate of 0.5 mL/min at 25 °C. Monodispersed poly(methyl methacrylate) standards were used to obtain a calibration curve. ^{1}H NMR spectra were measured by accumulation of 1000 scans at a relaxation time of 2 s on a Bruker ARX 300 MHz spectrometer, using $CDCl_3$ (for PGMA) and D_2O (for aminated PGMA) as the solvents. The chemical shifts were referred to the solvent peaks, δ = 7.20 ppm for $CDCl_3$ and δ = 4.70 ppm for D_2O, respectively.

Characterization of Polymer/pDNA 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 DNA (pDNA) was amplified in *Escherichia coli* and purified according to the supplier's protocol (Qiagen GmbH, Hilden, Germany). The purified pDNA was resuspended in Tris-EDTA (TE) buffer (containing 10 mM Tris-Cl and 1 mM EDTA), pH 7.4, and kept in aliquots of 0.5 mg/mL in concentration. All polymer stock solutions were prepared at a nitrogen concentration of 10 mM in distilled water. Solutions were filtered via sterile membranes of an average pore size of 0.2 μm and stored at 4 °C. Polymer to DNA ratios are expressed as molar ratios of nitrogen (N) in polycations to phosphate (P) in DNA (or as N/P ratios). The average mass weight of 325 per phosphate group of DNA was assumed. All polymer/pDNA complexes were formed by mixing equal volumes of polymer and pDNA solutions at the desired N/P ratio. Each mixture was vortexed and incubated for 30 min at room temperature. Each cationic polymer was examined for its ability to bind pDNA at various N/P ratios through agarose gel electrophoresis using the similar procedures as those described earlier.[8,9] Gel electrophoresis was carried out in the TEA running buffer (containing 40 mM Tris-acetate and 1 mM EDTA) at a voltage of 110 V for 30 min in a Sub-Cell system (Bio-Rad Lab, Hercules, CA). DNA bands were visualized and photographed by a UV transilluminator and BioDco-It imaging system (UVP Inc., Upland, CA). The particle sizes and zeta potentials of the polymer/pDNA complexes were measured in PBS in triplicate using a Zetasizer Nano ZS (Malvern Instruments, Southborough, MA). The polymer/pDNA complexes were also observed with the transmission electron microscopy (TEM) (JEOL JEM 2010F). In a typical experiment, one drop of the complex solution was introduced onto a copper grid. The droplet was allowed to be dried under reduced pressure and then observed under TEM operating at an acceleration voltage of 100 kV.

Determination of Buffering Capacity. The buffering capacity of cationic polymers in the pH range of 2–10 was determined by acid–base titration.[12] Polymers were dissolved into 20 mL of saline (0.9% NaCl solution) with a 10 mM amino group concentration, which was subsequently adjusted by 0.1 M NaOH to an initial pH 10. The solutions were titrated to pH 2 with a 0.1 N HCl solution with various volume increments. The pH of all the solutions was measured using a TOLEDO 320 pH meter (METTLER). PEI of the same amino group concentration was used as the control. Pure water was used as the blank solution.

Cell Viability. The cytotoxicity of the polymer vectors was evaluated using the MTT assay in HEK293 and COS7 cell lines. They were cultured in Dulbecco's modified eagle medium (DMEM), supplemented with 10% heat-inactivated fetal bovine serum (FBS), 100 units/mL of penicillin, and 100 μg/mL of streptomycin at 37 °C, under 5% CO_2, and 95% relative humidity atmosphere. The cells were seeded in a 96-well microtiter plate at a density of 10^4 cells/well and incubated in 100 μL of DMEM/well for 24 h. The culture media were replaced with fresh media containing serial dilutions of polymers or 10 μL of polymer/pDNA complex solution at various N/P ratios, and the cells were incubated for 24 h. Then, the culture media were replaced with fresh media containing MTT with a concentration of 0.5 mg/mL. After 5 h, the unreacted dye was removed by aspiration. The produced formazan crystals were dissolved in DMSO (100 μL/well). The absorbance was measured using a microplate reader (Bio-Rad 680, USA) at a wavelength of 570 nm. The cell viability (%) relative to control cells cultured in media without polymers was calculated from $[A]_{test}/[A]_{control} \times 100\%$, where $[A]_{test}$ and $[A]_{control}$ are the absorbance values of the wells (with the polymers) and control wells (without the polymers), respectively. For each sample, the final absorbance was the average of those measured from six wells in parallel.

In vitro Transfection Assay. Transfection assays were performed using plasmid pRL-CMV as the reporter gene in HEK293 and COS7 cell lines. In brief, the cells were seeded in 24-well plates at a density of 5×10^4 cells in 500 μL of medium/well and incubated for 24 h. The polymer/pDNA complexes at the N/P ratios from 10 to 35 were prepared a priori by adding the polymer solution into the DNA solution, followed by vortexing and incubation for 30 min at room temperature. At the time of transfection, the medium in each well was replaced with 300 μL of fresh normal serum medium (supplemented with 10% FBS). A 20 μL portion of complexes containing 1.0 μg of pDNA were added into each well and incubated with the cells for 4 h under the standard incubation conditions. Then, the medium was replaced with 500 μL of the fresh normal medium. The cells were further incubated for an additional 20 h under the same conditions, producing a total transfection time of 24 h. The cultured cells were washed with PBS twice, and lysed in 100 μL of the cell culture lysis reagent (Promega Co., Cergy Pontoise, France). Luciferase gene expression was quantified using a commercial kit (Promega Co., Cergy Pontoise, France) and a luminometer (Berthold Lumat LB 9507, Berthold Technologies GmbH. KG, Bad Wildbad, Germany). The protein concentration in the cell samples was analyzed using a bicinchoninic acid assay (Biorad Lab, Hercules, CA). Gene expression results were expressed as relative light units (RLUs) per milligram of cell protein lysate (RLU/mg protein). All experiments were repeated at least three times. The data were collected in triplicate and expressed as mean ± standard deviations.

Statistical Analysis. Data are presented as means ± standard deviation. Statistical significance ($p < 0.05$) was evaluated using the student *t*-test when only two groups were compared. If more than two groups were compared, evaluation of significance was performed using one-way analysis of variance (ANOVA) followed by Bonferroni's *post hoc test*. In all tests, statistical significance was set at $p < 0.05$.

RESULTS AND DISCUSSION

Preparation of Different Aminated PGMA Vectors. In this work, the different amine species (including AP1 for PGAP1, AP2 for PGAP2, EA for PGEA, EA/DED for PGEADED, DED for PGDED, and quaternized DED for QPGDED) functionalized PGMA vectors were prepared from the same starting PGMA (with about 105 GMA (M_w, 142 g/mol) repeat units, $M_n = 1.52 \times 10^4$ g/mol determined by GPC) using a large excess of different amine species (Figure 1). To ensure the complete functionalization of the epoxy groups, the molar ratio of amine species to GMA units was over 50 in the ring-opening reactions. In order to increase the surface cationic charges of pDNA complexes, PGDED was partially quaternized with bromoethane to produce QPGDED with quaternary ammonium groups. On the basis of the molecular weights of AP1 (75 g/mol), AP2 (75 g/mol), EA (61 g/mol), and DED (88 g/mol), the molecular weights of the corresponding PGAP1, PGAP2, PGEA, and PGDED from PGMA with about 105 GMA repeat units were estimated to be 2.31×10^4, 2.31×10^4, 2.16×10^4, and 2.44×10^4 g/mol, respectively.

The representative structures of PGMA, PGAP1, PGAP2, PGEA, PGEADED, PGDED, and QPGDED were characterized by ^{1}H NMR spectra (Figure S1 Supporting Information). For PGMA (Figure S1a), the signals at δ = 3.8 and 4.3 ppm corresponded to the methylene protons adjacent to the oxygen moieties of the ester linkages (**a**, $\underline{CH_2}$—O—C═O). The peaks at δ = 3.2 ppm and δ = 2.63 and 2.84 ppm were assigned to the CH_2—$\underline{CH}$(O)—CH_2 (**b**) methylidyne and CH—$\underline{CH}$(O)—CH_2 (**c**) methylene protons of the epoxide ring, respectively. The peak area ratio of **a**, **b**, and **c** was about 2:1:2, consistent with the molecular structure of PGMA. After the ring-opening

reactions of PGMA with AP1, AP2, EA, and DED (Figure S1b–f), the peaks (**b**, **c**) associated with the epoxy groups of PGMA disappeared completely, indicating that the almost all oxirane rings were opened. The peaks (**a**, $\underline{CH_2}$–O—C═O) at δ = 3.8 and 4.3 ppm shifted to one position (**d**, around 3.95 ppm). The new peak region located at chemical shifts of δ = 3.6–3.8 ppm was mainly associated with the methylidyne and methylene protons adjacent to the hydroxyl groups (**e**, $\underline{CH}$—OH and **e′**, $\underline{CH_2}$—OH). The signal at δ = 2.7 ppm was mainly attributable to the methylene protons adjacent to the amine groups (**f**, $\underline{CH_2}$—NH for PGAP1, PGAP2, and PGEA). For PGEADED and PGDED, the typical chemical shifts **f′**, **f″** at about 2.3–2.5 ppm were mainly attributable to the $\underline{CH_2}$–N and $\underline{CH_3}$–N species. On the basis of the area ratio of peaks **f′**, **f″** and peak **f**, the DED:EA molar ratio of PGEADED was estimated to be about 1:5. After quaternization, the new signals at about 3.2 and 3.5 ppm were attributable to the (**g**) N^+–$\underline{CH_3}$ methyl and (**h**) N^+–$\underline{CH_2}$ methylene protons of the quaternized species, respectively.[16,17] On the basis of the area ratio of peaks **g**, **h**, and peaks **f′**, **f″**, and **f**, the quaternization ratio of QPGDED was about 70%.

Biophysical Characterization of Cationic Vectors. A successful gene delivery system requires that plasmid DNA (pDNA) has to be condensed by polycation into nanoparticles small enough to facilitate cellular uptake. Thus, the pDNA condensation capability is a prerequisite for polymeric gene vectors. In this work, the abilities of different amine species-functionalized PGMA vectors to condense pDNA were first confirmed by agarose gel electrophoresis. Figure 2 shows the gel retardation results of various polymer/pDNA complexes with increasing nitrogen/phosphate (or N/P) ratios in comparison with that of branched PEI (25 kDa). PGEA, PGAP1, and PGAP2 exhibited similar condensation capabilities. They compacted pDNA completely at the N/P ratio of 3.0 and above, indicating that the additional methyl (from AP1) or methylene (from AP2) group did not obviously affect the DNA condensation capability. PGEADED and PGDED showed the decreased condensation ability, and they completely retarded the migration of pDNA at the N/P ratio of 4. The tertiary amine group of DED could not enhance the condensation ability. In comparison with PGDED (Figure 3e), QPGDED exhibited the enhanced condensation capability and inhibited the complete migration of pDNA at the N/P ratio of 2.5. It was reported that the quaternized tertiary amine group could enhance the surface cationic charges, producing higher pDNA condensation abilities.[16,17]

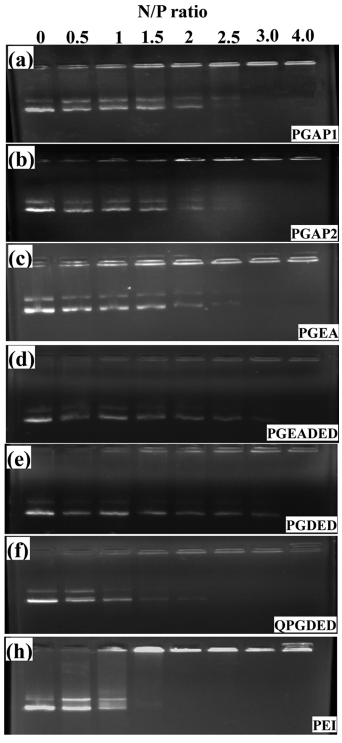

Figure 2. Electrophoretic mobility of plasmid DNA (pDNA) in the complexes of the cationic polymers ((a) PGAP1, (b) PGAP2, (c) PGEA, (d) PGEADED, (e) PGDED, (f) QPGDED, and (h) PEI) at various N/P ratios.

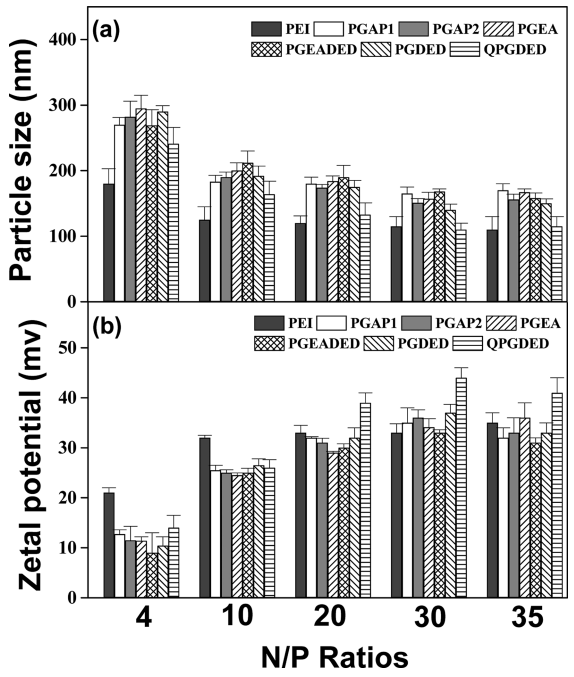

Figure 3. Particle size (a) and zeta potential (b) of the complexes between the cationic polymers (PGAP1, PGAP2, PGEA, PGEADED, PGDED, QPGED and PEI) and pDNA at various N/P ratios.

The particle size and surface charge of complexes are important factors in modulating cellular uptake. The (a) particle size and (b) zeta potential of the aminated PGMA vector/pDNA complexes were compared with those of the PEI/pDNA complexes at various N/P ratios in Figure 3. All the cationic polymers could efficiently compact pDNA into small particles (Figure 3a). At the N/P ratio of 4, large aggregates for all the complexes were formed, due to the lower content of cationic polymers. At higher N/P ratios, all vectors condensed pDNA into nanoparticles in the diameter range of 100–200 nm. The complex sizes of QPGDED/pDNA were lower than those of PGDED/pDNA at most N/P ratios, which was consistent with the higher condensation ability of QPGDED (Figure 3f). Figure 4 shows the representative TEM images of the (a) PGAP1/pDNA, (b) PGEA/pDNA, and (c) PGDED/pDNA complexes at a ratio of 20. The images clearly revealed that the compacted complexes displayed a characteristic globular morphology and existed in the form of spherical nanoparticles. Zeta potential is an indicator of surface charges on the polymer/pDNA nanoparticles. After reaching the N/P ratio of 10, the zeta potentials of the complexes of all the

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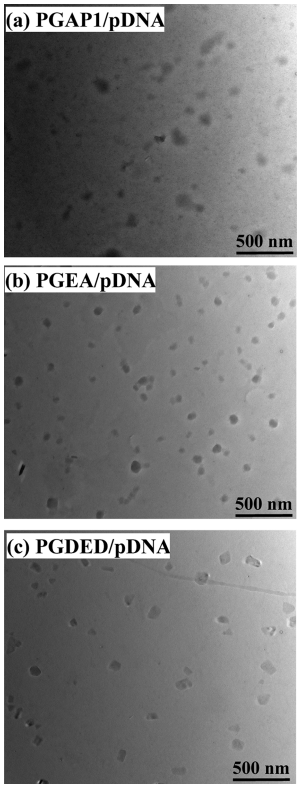

Figure 4. TEM images of the (a) PGAP1/pDNA, (b) PGEA/pDNA, and (c) PGDED/pDNA complexes at a ratio of 20.

cationic polymers were strongly positive (Figure 3b). A positively charged surface allows electrostatic interaction with anionic cell surfaces and facilitates cellular uptake. In comparison with PGDED/pDNA, QPGDED/pDNA exhibited higher cationic charges, which is expected to produce increasing affinity for anionic cell surfaces.

After cellular entry via endocytosis, the polymer/pDNA complexes were transported into lysosome. Efficient escape from endosomes is one of the most important factors to be considered for the design of gene delivery vehicles. This event is associated with the buffering capacity of gene vectors within the pH range of 7.4−5.0, where vectors undergo a change from the extracellular environment to the endosomal environment. By disrupting the endosomal membrane, polycations with high buffering capacity can mediate efficient escape from endosome to cytosol triggered by the acidic environment of endosome. In this study, acid−base titration under the given 10 mM amino group concentration was performed to evaluate the proton-buffering effects of PGMA-based vectors (Figure 5). PGAP1, PGAP2, and PGEA showed significantly higher buffering capacity than PEI (25 kDa), a well-known transfection agent due to its strong proton-sponge effect.[18] The local environment of the nonionic hydrophilic hydroxyl groups from the aminated PGMA vectors may benefit buffering capacities.[12] In comparison with PGEA, PGAP1 and PGAP2 exhibited slightly lower buffering capacities, probably due to the introduction of additional methyl (from AP1) or methylene group (from AP2). On the other hand, PGEADED, PGDED, and QPGDED showed significantly lower buffering capacities than PGEA. The above results indicated that the introduction of the tertiary group of DED or the quaternized DED seriously affected the buffering capacity.

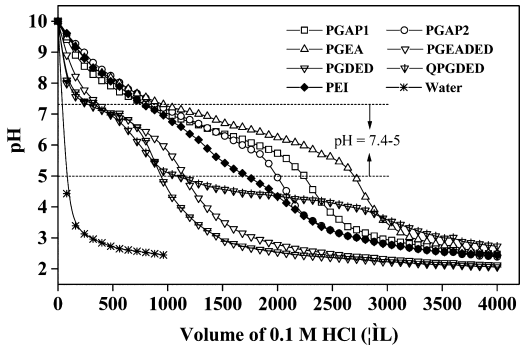

Figure 5. Determination of the buffer capacity of PGAP1, PGAP2, PGEA, PGEADED, PGDED, QPGDED, PEI, and water by acid−base titration. The cationic polymer solutions with 10 mM amino group concentration were titrated with 0.1 M HCl solution.

Cell Viability Assay. Cytotoxicity is another important factor to be considered in selecting polymeric gene carriers. Figure 6 shows the in vitro cell viabilities of PGAP1, PGAP2,

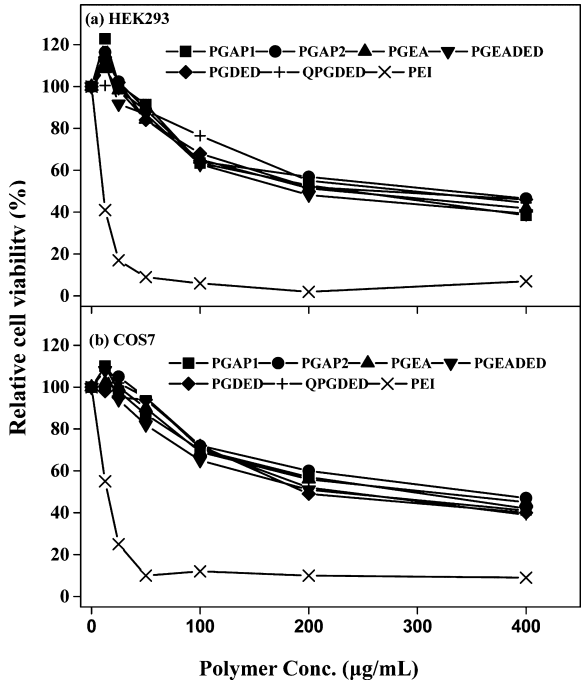

Figure 6. Cell viability assay in (a) HEK293 and (b) COS7 cells with various mass concentrations of PGAP1, PGAP2, PGEA, PGEADED, PGDED, QPGDED, and PEI (25 kDa).

PGEA, PGEADED, PGDED, QPGDED, and PEI (25 kDa) in HEK293 and COS7 cell lines. All of the cationic polymers exhibited a dose-dependent cytotoxicity effect. All the aminated PGMA vectors exhibited substantially lower toxicity than PEI. The slope of the dose-dependent cytotoxicity curve for PEI was sharply steeper. PEI is mainly composed of secondary amine groups. No obvious differences in cytotoxicity were observed among the aminated PGMA vectors, particularly for the tertiary amine group-containing vectors, PGEADED, PGDED, and QPGDED. It was reported that the introduction of quaternary ammonium groups could increase cytotoxicity.[16−18] In this work, no obvious change in cytotoxicity of QPGDED indicated that pendant nonionic hydrophilic hydroxyl units of the aminated PGMA vectors played a predominant role in

determining the cytotoxicities. The flanking hydroxyl groups probably well shielded the deleterious cationic charges of gene carriers.[12]

The cell viability of polymer/pDNA complexes as a function of the N/P ratio was also evaluated in the HEK293 and COS7 cell lines (Figure 7). The N/P ratio had a profound impact on

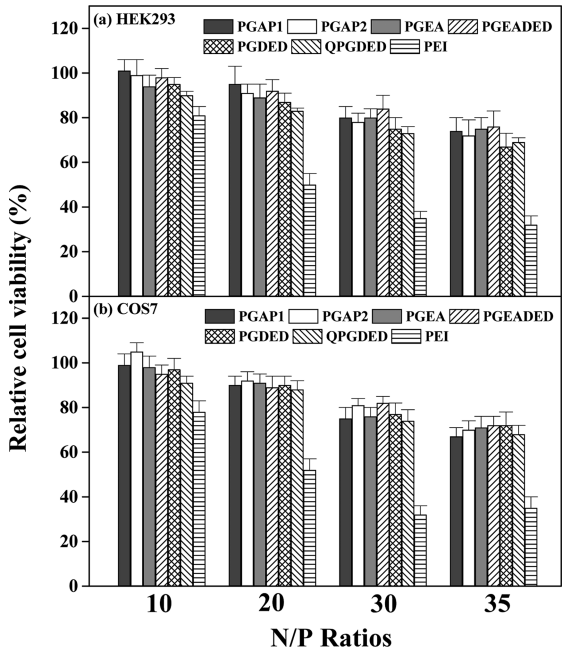

Figure 7. Cell viability assay in (a) HEK293 and (b) COS7 cells with various N/P ratios of PGAP1, PGAP2, PGEA, PGEADED, PGDED, QPGDED, and PEI (25 kDa).

the cytotoxicity of complexes. The cell viability of all the polymer/pDNA complexes decreased with increasing N/P ratios. At higher N/P ratios, the transfection formulation also contained free polymers, besides the compact and positively charged polymer/pDNA complexes. The increased free cationic polymers would increase cytotoxicity. At the same N/P ratio, no obvious differences were observed among the aminated PGMA/pDNA complexes, where the control PEI/pDNA complexes exhibited much higher cytotoxicity. The above results further confirmed the importance of the flanking hydroxyl units of the aminated PGMA vectors in reducing cytotoxicity.

In vitro Gene Transfection Assay. The in vitro gene transfection efficiencies of the aminated PGMA/pDNA complexes were assessed using luciferase as a gene reporter in HEK293 and COS7 cell lines. Figure 8 shows the gene transfection efficiencies mediated by the aminated PGMA vectors at various N/P ratios in the presence of serum in comparison with that of "gold standard" PEI (25 kDa) at the N/P ratio of 10, where branched PEI usually exhibited the highest transfection efficiency.[12−14] PGAP1 exhibited higher transfection efficiencies than PGEA at all N/P ratios. At lower N/P ratios, no obvious differences between the transfection efficiencies mediated by PGAP2 and PGEA were observed, while at higher N/P ratios, PGAP2 produced higher efficiencies than PGEA. In comparison with EA, AP1 (or AP2) contains an additional methyl (or methylene) group. The above results suggested that the introduced methyl or methylene species could benefit the gene delivery. As discussed above, PGEA,

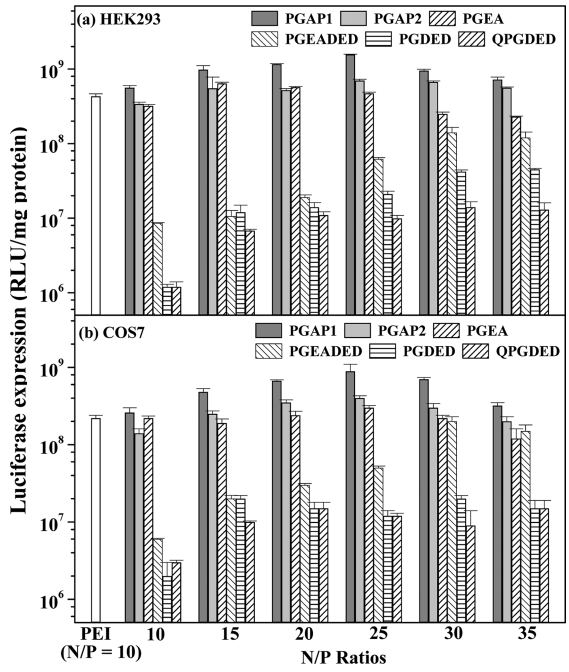

Figure 8. In vitro gene transfection efficiency of the cationic polymers (PGAP1, PGAP2, PGEA, PGEADED, PGDED, and QPGDED)/pDNA complexes at various N/P ratios in comparison with that of branched PEI (25) at its optimal N/P ratio of 10 in the presence of serum in (a) HEK293 and (b) COS7 cells.

PGAP1, and PGAP2 exhibited similar condensation capabilities (Figure 2). PGAP1 and PGAP2 exhibited slightly lower buffering capacities than PGEA (Figure 5). The results indicated that the efficient gene delivery from PGAP1 and PGAP2 was due to other beneficial factors. The hydrophobic−hydrophilic balance of the carrier system is another important factor for gene delivery. The suitable incorporation of the hydrophobic species into the gene vectors could enhance gene transfection efficiency.[19,20] The hydrophobic nature induced by the additional methyl or methylene group might increase the interaction between the cationic vector/pDNA complexes and cells by modulating the adsorption on cell surfaces and cell uptake. In addition, the better transfection performance of PGAP1 than PGAP2 indicated that the enhancement of transfection efficiency also depended on the local position of the hydrophobic group.

With the increase in the DED content, PGEADED and PGDED showed the substantial decrease in transfection efficiencies (Figure 8). DED possesses a tertiary amine group, which slightly decreased the pDNA condensation ability (Figure 2). In addition, PGEADED and PGDED exhibited significantly lower pH buffering capacities (Figure 5), which may take the major responsibility for their poor transfection abilities. The lower buffering capacity probably prevented the plamid escape from the cationic vector/pDNA complexes in endosomes after cellular entry. Upon the quaternization, the quaternized tertiary amine groups of QPGDED could enhance the surface cationic charge and pDNA condensation ability (Figures 2 and 3), which should provide increased affinities for anionic cell surfaces and benefit cellular uptake. However, such positive physiochemical factors did not produce higher transfection efficiencies. As shown in Figure 8, QPGDED exhibited the lowest transfection efficiencies at most N/P ratios,

probably due to its worst buffering capacity within the pH range of 7.4−5 (Figure 5).

CONCLUSIONS

Different aminated PGMA vectors by AP1, AP2, EA, EA/DED, DED, and quaternized DED were systematically compared in terms of the condensation abilities, pH buffering capacities, cytotoxicities, and gene transfection efficiencies. The additional methyl (from AP1) or methylene (from AP2) group did not significantly affect pH buffering capacities. But the methyl or methylene species could benefit the gene delivery, and the transfection performance mediated by AP1-aminated PGMA is best. The tertiary amine group of DED could be quaternized to enhance the surface cationic charge and pDNA condensation ability. However, both DED and its quaternized counterpart (QDED) seriously affected buffering capacity, making the DED- or QDED-containing vectors exhibit poor gene transfection performances. Thus, structural tailoring of aminated PGMA vectors could provide a versatile approach for constructing better gene-delivery systems with good biophysical properties.

ASSOCIATED CONTENT

Supporting Information

^{1}H NMR spectra of the functionalized PGMA vectors. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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