Synthesis and Evaluation of Phenylalanine-Modified Hyperbranched Poly(amido amine)s as Promising Gene Carriers

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Hyperbranched poly(amido amine) (HPAMAM), which is structurally analogous to PAMAM dendrimers, has been proposed to be an effective agent for gene delivery. The facile synthesis of HPAMAM with scalable productivity by one-pot polymerization of monomers of methyl acrylate (MA) and diethylenetriamine (DETA) has been set up previously. In this study, the HPAMAM was further modified on the terminal amino groups with phenylalanine to various degrees (HPAMAM-PHE30, PHE45, PHE60). We showed that HPAMAM and HPAMAM-PHEs were all able to form complexes with plasmid DNA (pDNA) at various mass ratios. The cytotoxicity and transfection efficiencies of these polymers were evaluated in SMMC-7721 and COS-7 cell lines. The PHE modifications affected the cell transfection efficiency significantly. The HPAMAM-PHE60 was the most efficient, with transfection activities consistently higher than the commercial transfection reagent PEI. Our study demonstrated that HPAMAM-PHEs may be good new materials for gene delivery and other applications because of its large-scale availability, economical cost, and low toxicity.

1. Introduction

Gene therapy represents a new and promising method for disease treatment by transmission of DNA that encodes therapeutic genes and/or consequent expression of therapeutically active proteins. Several cationic polymers have been developed and found to be efficient for cell transfection, including polyethylenimine (PEI)[1,2] and starburst poly(amido amine) (PAMAM) dendrimers.[3−6] They have shown high and consistent transfection against many cell lines,[7,8] but their in vivo applications have been limited for reasons such as poor specificity and significant toxicity. Therefore, it is important to improve and develop new cationic polymers for future gene therapy applications.

Starburst dendritic or hyperbranched polymers have attracted a lot of attention lately.[9−16] The PAMAM dendrimer is a landmark molecule that has a well-defined globular structure and high density of surface positive charges that can condense DNA tightly and interact with cells. It showed remarkable gene delivery efficiencies in vitro and is considered to have great potential in many drug and gene delivery applications.[17−20] Some recent studies had further modified the dendrimer surface using PEG, β-cyclodextrins, or L-arginine and reported improvements,[21−24] but because the synthetic procedure of dendrimer itself is already complex, surface modification would introduce more steps and limit large-scale production and application.[25]

Hyperbranched polymers[26−38] were structurally analogous to dendrimers and have similar architecture and properties in terms of globular topology, low viscosity, high solubility, and high density of functional groups, whereas they can be obtained by a simpler and more economical one-step/pot polymerization technique. Wu et al. prepared hyperbranched PAMAM (HPAMAM) by polycondensation of *N*-[2-(1-piperazinyl)ethyl]-1,2-ethanediamine (AEPZ) and maleic anhydride (BDA), and the resulting HPAMAM showed good DNA protect ability and high gene transfection efficiency.[39,40] Gao and coworkers previously developed a new method of synthesizing HPAMAM by polycondensation of methyl acrylate (MA) and diethylenetriamine (DETA).[41,42] It has very similar unit structure to the classic PAMAM dendrimer but a much simpler synthesis procedure. In this article, we used the HPAMAM and phenylalanine-modified HPAMAM as a new kind of polycation to explore their bioapplications, and we found that these HPAMAMs have significant gene transfection activities; even more significant, the phenylalanine modification could improve the gene transfection efficiency significantly without much of an increase in toxicity.

2. Experimental Section

2.1. Materials. For the synthesis of HPMAMs, MA (Alfa), DETA (Aldrich), and tertiary butylhydroquinone (TBHQ, Acros) were purchased with the highest purity available and used without further purification. The resulting polymer molecular weights were confirmed using gel permeation chromatography (GPC, PE series 200) with PS as standards and DMF as the eluent at a flow rate of 1 mL/min. The structure characteristics were confirmed by ^{1}H NMR spectra, obtained using a Varian VPX 300 MHz spectrometer. BOC-phenylalanine (BOC-D-PHE) and DNase I were purchased from Sigma-Aldrich (St. Louis, MO). 4-Dimethylamino-pyridine (DMAP) and 1,3-dicyclohexylcarbodiimide (DCC) were purchased from Shanghai Medpep. Chloroform ($CHCl_3$) was dried by refluxing over CaH_2 and was distilled prior to use. Branched PEI (MW = 25 kDa, Aldrich) was used as a control transfection agent. D_2O used as the solvent in the NMR measurements was obtained from Aldrich. For cell transfection studies, 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) was

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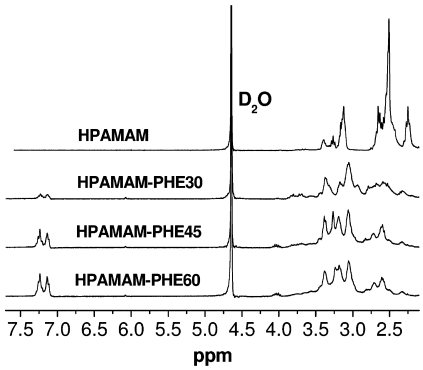


Figure 1. ^{1}H NMR spectra of HPAMAM, HPAMAM-PHE30, HPAMAM-PHE45, and HPAMAM-PHE60 in D_2O.

purchased from Ken-Real (Shanghai). The pGL_3 vector (pDNA, 5256 bp) encoding the luciferase reporter gene was purchased from Promega (Madison, WI). Fetal bovine serum (FBS) and RPMI1640 medium were obtained from GIBCO (Gaithersburg, MD). The luciferase assay kit was purchased from Promega (Madison, WI).

2.2. Synthesis of HPAMAMs. HPAMAMs were synthesized from MA and DETA according to the published protocol via "couple-monomer methodology".[41−43] The used HPAMAM had a number-average molecular weight (M_n) of 5510 and polydispersity index (PDI) of 1.86. We also synthesized another batch of HPAMAM (HPAMAM-2) with M_n of 11 500 and PDI of 1.88 for the comparison study.

2.3. Synthesis of Phenylalanine-Modified HPAMAM. The HPAMAM polymers were further modified by conjugating phenylalanine residues to the surface amino groups. In typical, HPAMAM (1.5 g, ca. 14 mmol amino groups) was dissolved in anhydrous chloroform (50 mL) in a flask under nitrogen gas protection. Then, DCC and Boc-PHE with specific molar percentages (30, 45, and 60%) in terms of amino groups of HPAMAM were added together with DMAP (0.244 g) to the flask that was immersed in an ice−water bath. The mixture was stirred at 0−5 °C for 3 h and then remained at room temperature overnight. After the solid of dicyclohexylurea (DCU) was removed by filtration and centrifugation, the solution was precipitated by the addition of diethyl ether, and Boc-PHE-modified HPAMAM was obtained. To deprotect the *tert*-butoxycarbonyl (BOC) groups, we mixed the as-obtained product with 1 M HCl aqueous solution and stirred for 2 h at 50 °C. The deprotected phenylalanine-modified HPAMAM (HPAMAM-PHE) was collected by precipitation with acetone, followed by freeze-drying.

The resulting polymers were confirmed by ^{1}H NMR measurements. (See Figure 1.) ^{1}H NMR (300 MHz, D_2O), HPAMAM-PHE-60: 2.2−3.8 (branched, protons of HPAMAM and $-CH_2CH-$ of PHE unit), 4.05 (w, $-CH(NH_2)-$ of PHE unit), 7.1−7.4 (s, benzene protons of PHE unit). According to the integration ratio of the protons at 2.2−3.8 to 7.1−7.4, the conversions of amino groups for the samples of HPAMAM-PHE30, HPAMAM-PHE45, and HPAMAM-PHE60 were 9, 15, and 21%, respectively.

2.4. Cell Lines, Cell Culture. The human hepatocarcinoma cell line SMMC-7721 and African green monkey kidney cell line COS-7 were cultured in RPMI1640 supplemented with 10% FBS (Logan, Utah), 100 U/mg streptomycin, and 100 U/mL penicillin. All cells were maintained at 37 °C in humidified 5% CO_2.

2.5. Preparation of Polymer/DNA Complexes. Complexes were prepared at different polymer/DNA mass ratios by the addition of the polymer aqueous solution to equal volumes of calf thymus DNA (Sigma) (for size and ζ-potential measurements) or plasmid DNA (for in vitro GFP and luciferase expression studies) solutions with gentle vortexing (Ortex2 Genie oscillator scientific industries company) and incubating at room temperature for 30 min.

2.6. Gel Retardation Assay. To confirm and compare DNA condensation ability of the polymers, we performed gel electrophoresis. Complexes formed at various mass ratios were loaded onto 1% agarose gels with ethidium bromide (EtBr) (0.1 μg/mL) and run with tris-acetate (TAE) running buffer at 80 V for 45 min. DNA retardation was observed and photographed by irradiation with UV transilluminator and recorded with Cam2com software.

2.7. Measurement of Particle Size and ζ Potential. The size and surface charge of the polymer/DNA complexes were measured using a dynamic light scattering spectrophotometer (Zetasizer 3000HSA, Malvern Instrument) at 25 °C. The volume of each sample was 2 mL, containing a DNA concentration of 0.1 μg/μL. ζ potentials were measured in purified water (Zetasizer Nano ZS, Malvern Instrument).

2.8. Cell Transfection − Luciferase Activity Assay. Cells were seeded in 24-well or 48-well plates at appropriate density and grown in complete culture medium for 18−24 h to reach 50−70% confluence at the time of transfection. Before transfection, the medium was replaced with serum-free medium. The prepared complexes (1 μg DNA/well in 24-well plates and 0.5 μgDNA/well in 48-well plates) with various mass ratios were added to the culture medium and incubated with cells for 4 h under standard incubator conditions. After that, the medium was replaced with fresh medium containing 10% FBS, and the cells were further incubated for an additional 24 h. For assaying luciferase activity, cells were washed with PBS twice, and 100−200 μL of cell lysis buffer (1 mM) was added for 5 min at 37 °C. The lysate was centrifuged at 12 000 rpm for 5 min. Supernatant (10 μL) was mixed with 25 μL of luciferase substrate and 25 μL of ATP solution, and the luciferase activity was measured by Sirius luminometer (Autolumat LB953, EG and G, Berthold, Germany). Protein quantification was determined by the bicinchoninic acid assay (Bio-Rad Laboratories, Hercules, CA), and RLUs were normalized to protein concentration in the cell extracts. Each transfection experiment was carried out in triplicate, and transfection activity was expressed as relative light units. The branched PEI (MW = 25 kDa) was used in every experiment for comparison.

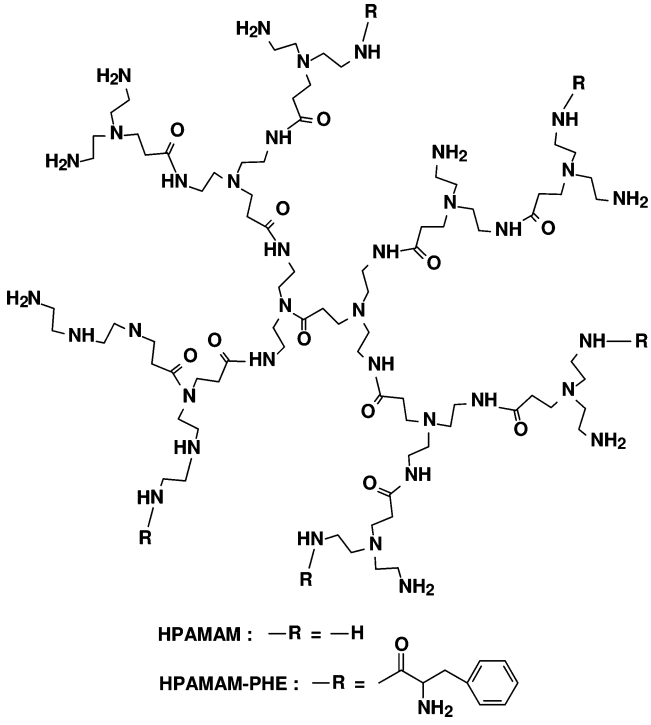
2.9. Cell Transfection-Green Fluorescence Protein Expression. SMMC-7721 cells were seeded in a six-well plate at the density of 1.0×10^6 in 2 mL of culture medium for 18 h (to reach 50% confluence at the time of transfection). Before transfection, the medium was replaced with serum-free medium. Then, the cells were treated with polymer/DNA complexes (DNA dose 2 μg/well) at specific mass ratios for 4 h. Afterward, the medium was replaced by fresh medium containing 10% serum, and the cells were further incubated for 24 h. Green fluorescent protein (GFP) expression was visualized using fluorescence microscopy (OLYMPUS IX71, Japan).

2.10. Cytotoxicity Assay Using MTT. The cytotoxicities of HPAMAMs were examined using cell viability assay on the basis of MTT in SMMC-7721 and COS-7 cell lines. Cells were seeded in 96-well plates at an initial density of 1×10^4 cells/well and cultured for 24 h at 37 °C, 5% CO_2, and 95% relative humidity. After the cells reach 50−70% confluence, the growth media was replaced by fresh, serum-free media containing various amounts of polymers and polymer/DNA (0.4 μg DNA/well). After incubation for 4 h, the media were changed back to growth media and incubated for 24 h. Then, 10 μL of sterile-filtered MTT stock solution in PBS (5 mg/mL) was added to each well, reaching a final MTT concentration of 0.5 mg/mL. After 4 h, the residual dye was removed by aspiration. The formazan crystals were dissolved in DMSO (100 μL/well) and shaken evenly for 10 min. The absorbance was measured at 570 nm using an ELISA plate reader (MOBEL 550, Bio-Rad). Cell viability (%) was calculated according to the following equation: relative cell viability = OD570 (sample)/OD570 (control), where OD570 (control) represents the measurement from a well treated with cell culture medium only. All experiments were conducted for five samples and averaged.

3. Results

3.1. Synthesis of HPAMAM and HPAMAM-PHE. Scheme 1 shows the chemical structure of HPAMAM and HPAMAM-PHE. The HPAMAM has a structure that is very similar to that of the PAMAM dendrimer. HPAMAM-PHE was synthesized

Scheme 1. Chemical Structures of Hyperbranched Poly(amido amine) (HPAMAM) and HPAMAM Modified with Phenylalanine (HPAMAM-PHE)



to contain different ratios of PHE conjugation of the surface amino groups. To facilitate the illustration in the context, we named the phenylalanine-modified polymers as HPAMAM-PHE30, HPAMAM-PHE45, HPAMAM-PHE60 in terms of feed molar ratios.

3.2. Characterization of the HPAMAM/DNA Complexes. The DNA condensation capabilities of the HPAMAM and HPMAM-PHE polymers were evaluated by agarose gel electrophoresis. Figure 2 shows the gel retardation results of complexes with increasing mass ratios.

The HPAMAM polymer can form complex with DNA effectively at mass ratios as low as 0.6/1 (N/P ratio, ca. 2/1), whereas its PHE-modified products were a little less potent and inhibited the migration of pDNA at mass ratios >1. The attachment of phenylalanine residues to HPAMAM decreased the surface charge densities, so a little more polymers were needed to condense DNA.

Figures 3 and 4 show the various HPAMAM/DNA complex sizes and ζ potentials. The HPAMAM/DNA and HPAMAM-PHE/DNA complexes were all well compacted with average sizes around 50−300 nm. The complex sizes were a little bigger when the polymer/DNA weight ratios were between 0.5 and 2. At mass ratios >3 (N/P ratio, ca. 10/1), the particle sizes were all quite small.

Figure 4 shows the ζ potentials of various HPAMAM/DNA and HPAMAM-PHE/DNA complexes. The surface potentials of the complexes all became positive at polymer/DNA mass ratios >3. The differences between different PHE modified polymers were not very significant, but they all behaved differently from the original HPAMAM. The ζ potentials of HPAMAM/DNA complexes were relatively low in purified water.

3.3. Cytotoxicity of HPAMAM/DNA Complexes. We examined the toxicity of the HPAMAM/DNA and HPAMAM-PHE/DNA complexes in the SMMC-7721 and COS-7 cells using MTT assays. We chose two representative mass ratio

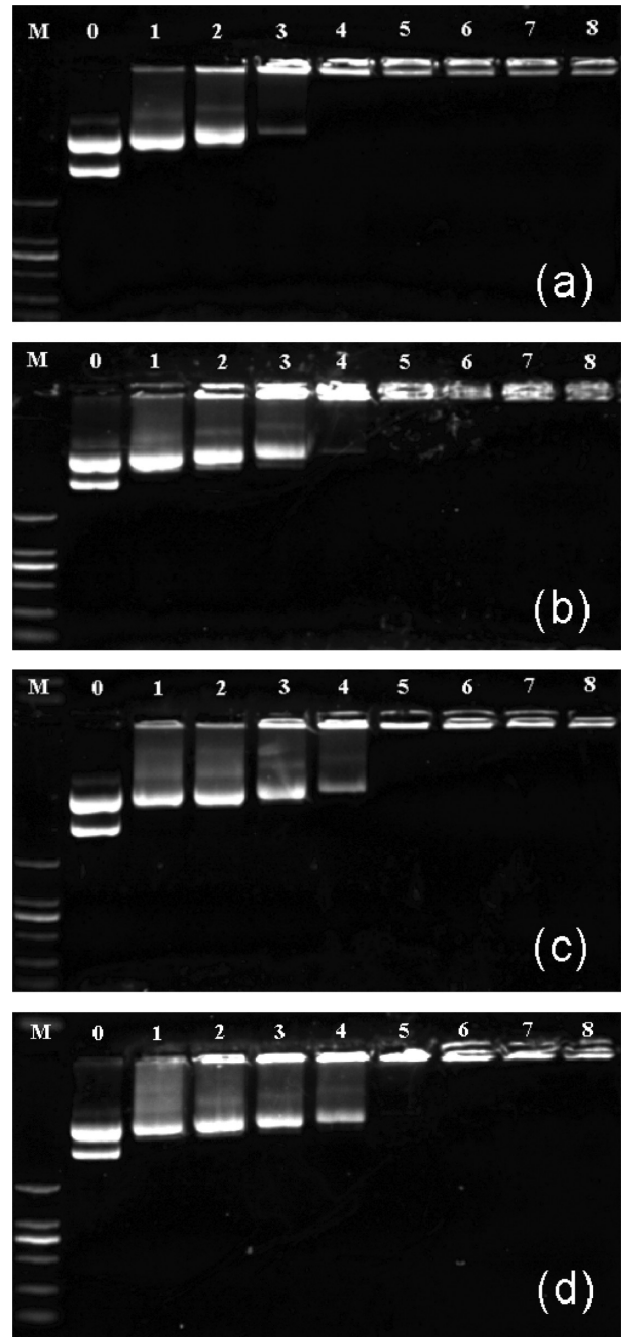


Figure 2. Agarose gel electrophoresis of HPAMAMs/DNA complexes: (a) HPAMAM, (b) HPAMAM-PHE30, (c) HPAMAM-PHE45, and (d) HPAMAM-PHE60. The mass ratios of polymer/DNA are 0.1, 0.3, 0.6, 1, 2, 3, 4, and 5 for lanes 1, 2, 3, 4, 5, 6, 7, and 8, respectively; Lanes M and 0 are marker (DL 2000, TaKaRa) and pDNA (~5000 bp).

complexes for the comparison and plotted the data in Figure 5. The cell treatment conditions were the same as those in transfection studies, but the DNA doses (0.4 μg/well) were all doubled. The cytotoxicities of the commercial transfection agent PEI were also evaluated for comparison. In general, the HPAMAM-PHE and HPAMAM all had only marginal cytotoxicities in SMMC-7721 and COS-7 cells. In contrast, the toxicity resulted from PEI was more obvious, and it increased sharply with the polymer concentration as well.

3.4. Cell Transfection Assay. The cell transfection efficiencies of the various polymer/DNA complexes were examined in both human hepatoma SMMC-7721 cells and African green

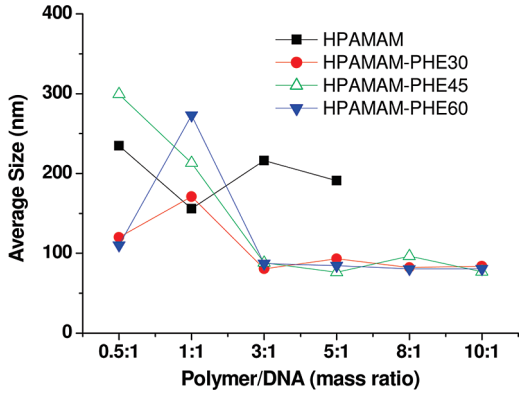


Figure 3. Average effective diameter of HPAMAM and HPAMAM-PHEs as a function of the hyperbranched polymer/DNA mass ratio (w/w).

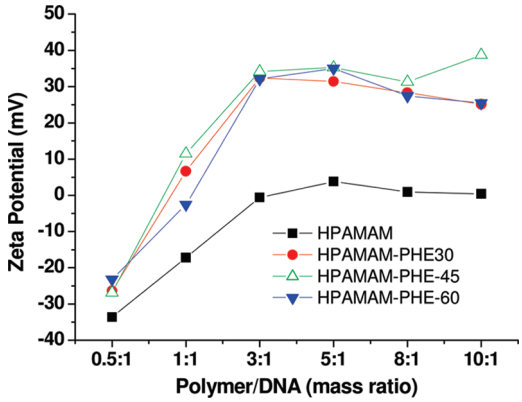


Figure 4. ζ potentials of HPAMAM/DNA and HPAMAM-PHE/DNA complexes as a function of the hyperbranched polymers/DNA mass ratio (w/w).

monkey kidney COS-7 cells. The luciferase reporter gene expression was evaluated quantitatively and reported in Figures 6 and 7. The PEI (25 kDa)/DNA complexes (mass ratio 5:1, N/P:15/1), which were frequently used in some cell transfection studies and reported to be the highest transfection activity,[3] were also tested for comparison as the control.

As shown in Figures 6A and 7A, HPAMAM/DNA complexes have good performance in both SMMC-7721 and COS-7 cells peaked at 12:1 and 9:1, which were even better than PEI control. PHE-modified products of HPAMAM also showed that good performance peaked at the mass ratios of about 9:1 to 18:1 (N/P, ca. 30/1 to 60/1) in different transfection assays. And with the increase in the PHE-modification ratio, the polymer showed much better performance in both SMMC-7721 and COS-7 cells than the PEI control (Figures 6B−D and 7B−D), which demonstrated that PHE-modification could improve the transfection efficiency of HPAMAM efficiently.

We also confirmed the efficient transfection by HPAMAM-PHE polymers using EGFP transgene expression studies. Figure 8 shows the fluorescence images of SMMC-7721 cells after transfection by HPAMAM/DNA (W/W 10/1), HPAMAM-PHE30/DNA (W/W 10/1), HPAMAM-PHE45/DNA (W/W 10/1), and HPAMAM-PHE60/DNA (W/W 12/1) complexes. Relatively strong fluorescence signals were observed when the transfection was mediated by the HPAMAM-PHE45/DNA and HPAMAM60-PHE/DNA complexes.

4. Discussions

PAMAM dendrimers are important transfection reagents that have been widely used. We synthesized a hyperbranched

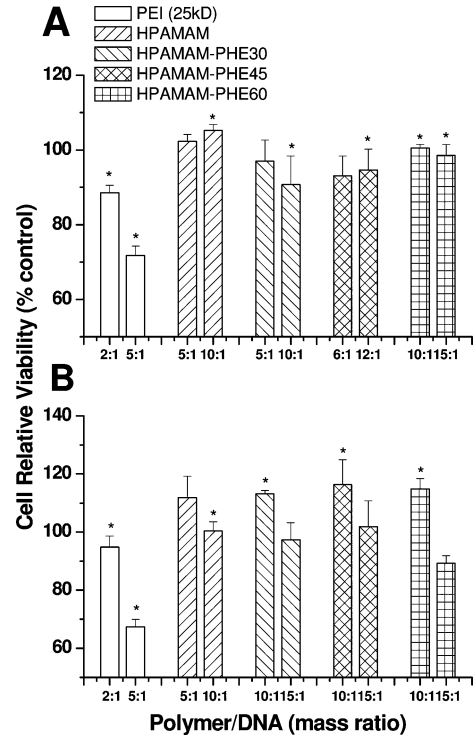


Figure 5. (A) SMMC-7721 and (B) COS-7 cells viability after incubating with HPAMAM/DNA and HPAMAM-PHEs/DNA complexes at different polymer/plasmid DNA mass ratios, as determined by the MTT assay. For comparison, the cell viability data of PEI (25 kDa)/DNA complexes were also given. Data were presented as mean (±SD, $n = 5$). * indicated $p < 0.05$ versus control group.

PAMAM from one-pot polymerization of commercial monomers of MA and DETA. The synthesis procedure is much simpler and faster.[41] The resulting HPAMAM has a similar molecular weight as a G3 PAMAM dendrimer (~6900) and many active terminal amino groups. Compared with the perfectly aligned dendrimer structure, the hyperbranched polymers would have better flexibility and may have actually resulted in better DNA complexation and gene transfection efficiencies. Krämer et al., in their studies, compared PEIs with different degrees of branching (DB) and reported that the best transfection was achieved using polymer at ~60% DB.[44] An even earlier and systematic study by Tang and Szoka had also pointed out that partial degradation of the dendrimer structure could improve the cell transfection efficiency, and the polymer chain flexibility is the key factor.[45] In our study, the synthesized HPAMAM was quite effective in transfection, and its cytotoxicity was low, suggesting that it may be a more economical alternative to starburst dendritic PAMAMs.

We further synthesized phenylalanine-modified HPAMAM by conjugating PHE to some of the HPAMAM terminal amino groups to improve its bioactivity further. A similar approach has been reported by Kono et al.[46] They conjugated PHE to a G4 PAMAM dendrimer and reported higher transfection efficiency and lower cytotoxicity after the conjugation. They proposed that the improvement in transfection was achieved through the synergy of the proton sponge effect, which is induced by the internal tertiary amines in the polymer and hydrophobic interaction by the hydrophobic amino acid residues on the dendrimer surfaces.[46] Shibata et al. also reported that a random copolymer of L-lysine and L-phenylalanine could translocate through phospholipids membranes much faster than

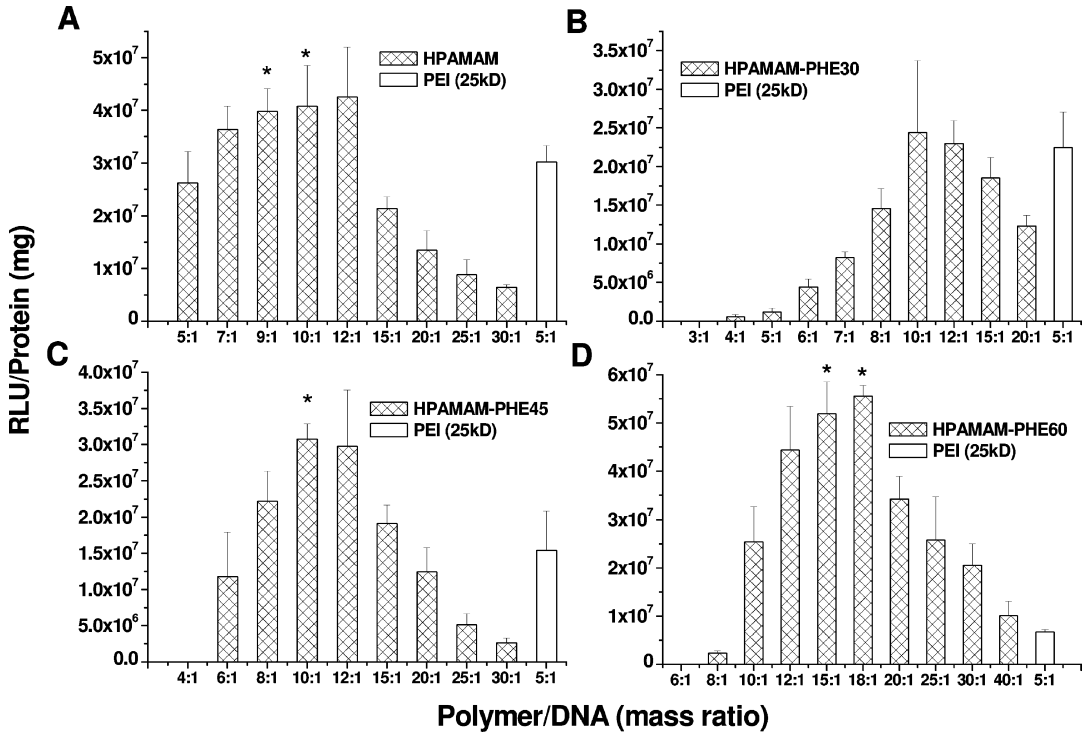


Figure 6. Transfection efficiencies (luciferase activities) of polyplexes based on HPAMAM and HPAMAM-PHE in SMMC-7721 cells using various polymer/plasmid DNA ratios (w/w). For comparison, the transfection efficiency using 25 kDa branched PEI (PEI/DNA 5:1 w/w) is also shown. The data were expressed as mean values of three experiments. * indicated $p < 0.05$ versus PEI control.

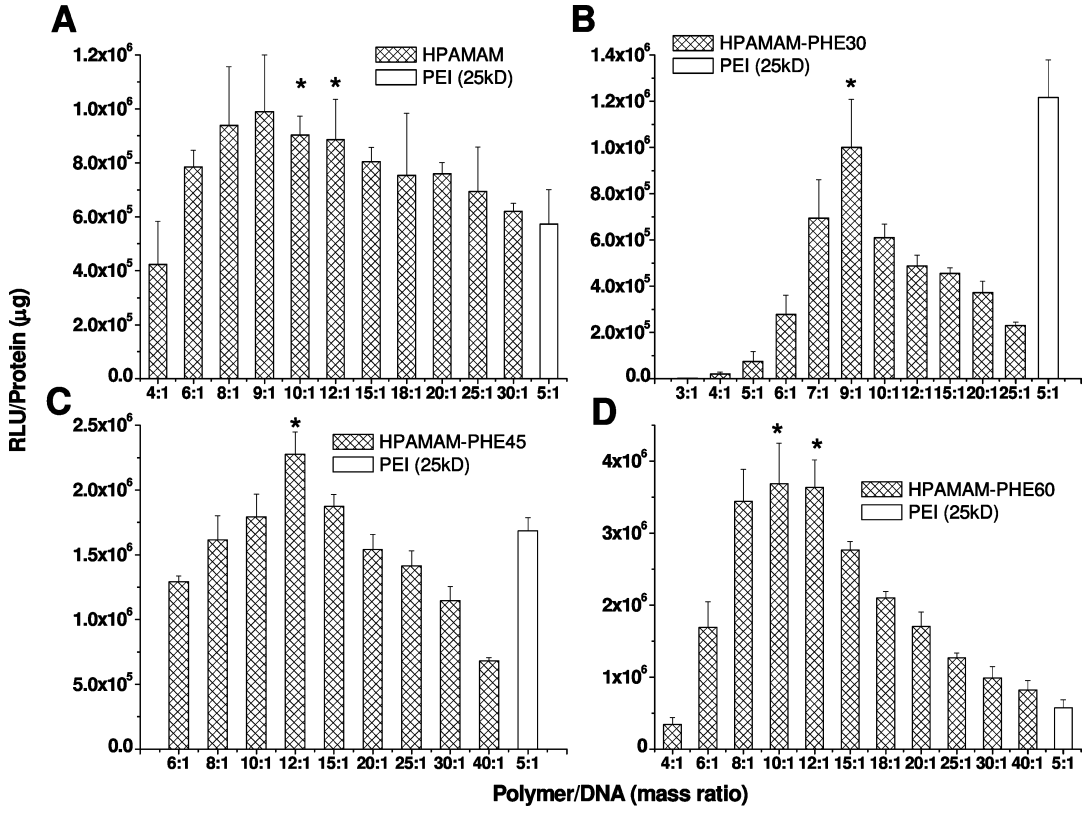


Figure 7. Transfection efficiencies (luciferase activities) of polyplexes based on HPAMAM and HPAMAM-PHE in COS-7 cells using various polymer/plasmid DNA ratios (w/w). For comparison, the transfection efficiency using 25 kDa branched PEI (PEI/DNA 5:1 w/w) is also given. The data were expressed as mean values of three experiments. * indicated $p < 0.05$ versus PEI control.

homopoly(L-lysine).[47] They suggested that the hydrophobic phenylalanine residue was responsible for the transmembrane activity.

In this study, we synthesized HPAMAM-PHE30, -PHE45, and -PHE60 polymers. The PHE modification ratios were the estimated molar amount of PHE added to the surface amino groups. On the basis of NMR measurements, the replacement ratios were actually conjugated at about 9, 15, and 21% ratios. The phenylalanine residues may form a hydrophobic environment in the periphery of the HPAMAM, reducing protonation

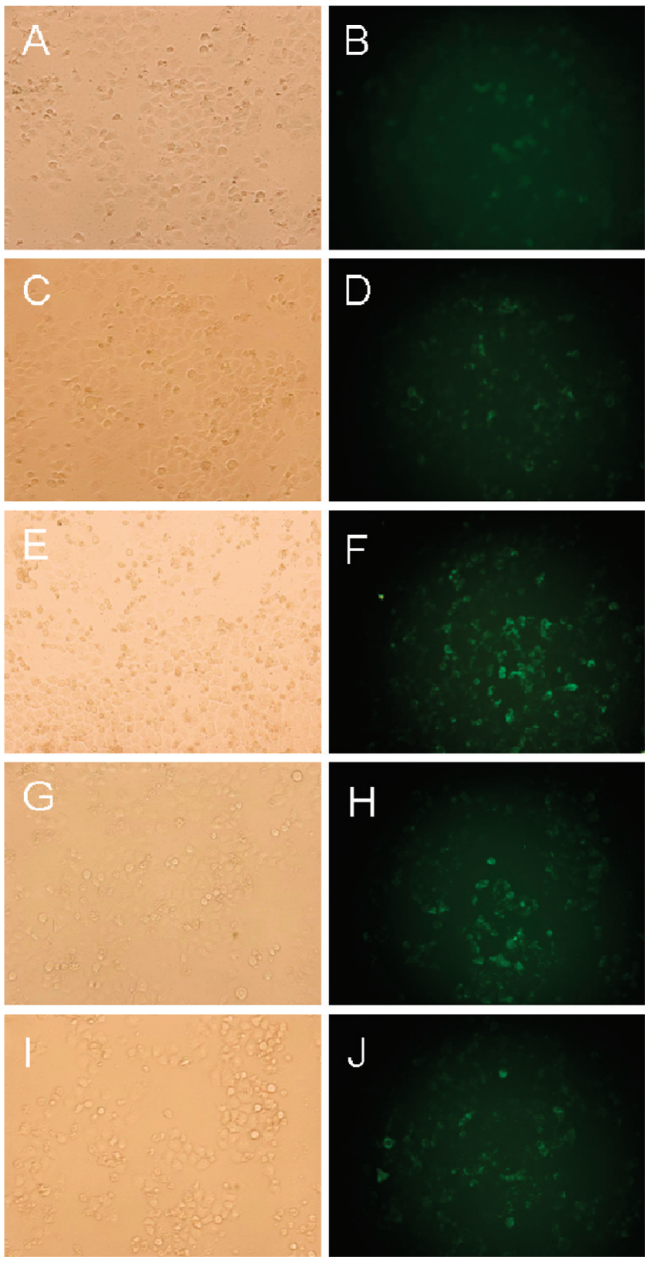


Figure 8. Fluorescence micrographs of GFP transgene expression in SMMC-7721 cells with polymer/DNA complexes transfected with (A,B) PEI (5/1 w/w), (C,D) HPAMAM (10/1 w/w), (E,F) HPAMAM-PHE30 (10/1 w/w), (G,H) HPAMAM-PHE45 (10/1 w/w), (I,J) HPAMAM-PHE60 (12/1 w/w), respectively. All mass ratios were chosen according to the luciferase assay.

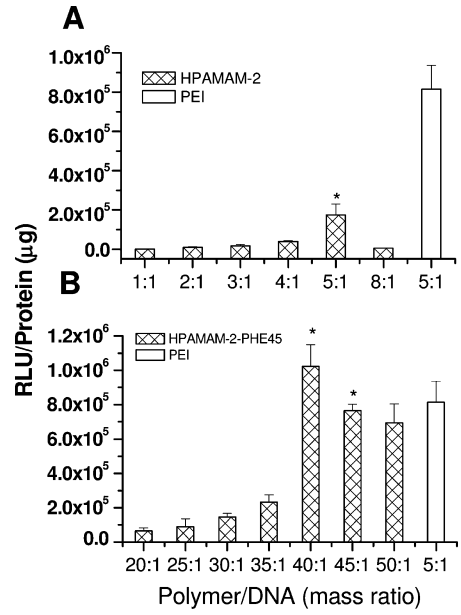


Figure 9. Transfection efficiencies (luciferase activities) of polyplexes based on (A) HPAMAM-2 and (B) HPAMAM-2-PHE45 in SMMC-7721 cells using various polymer/plasmid DNA ratios (w/w). For comparison, the transfection efficiency using 25 kDa branched PEI (PEI/DNA 5:1 w/w) is also given. The data were expressed as mean values of three experiments, * indicated $p < 0.05$ versus PEI control.

of their α-amino groups. With the increase in PHE modification, the best mass ratios for transfection become higher. Therefore, the HPAMAM-PHE showed a little weaker ability to form the complex with DNA than HPAMAM. Similar phenomenon had been observed for phenylalanine-modified PAMAM dendrimer.[48,49]

For gene delivery to cells, we were very glad to find that HPAMAM-PHEs could further improve transfection efficiencies. As shown in Figures 6 and 7, the improvement in transgene luciferase expression increased with the increase in surface PHE modification ratio compared with PEIs (25 kDa). The best transfection activity results were obtained using the HPAMAM-PHE60 polymer; the resulting transgene expression was almost one order of magnitude higher than PEI. Another clear advantage of the HPAMAM-PHEs polymer is their low cytotoxicity. The HPAMAMs obtained using our synthesis method were not very toxic to cells by themselves (Figure 5).

The molecular weight of the hyperbranched polymers could also affect the DNA complexation properties of HPAMAMs. It has been reported that PAMAM dendrimers with generation 5 and up are more effective for transfection.[50] However, this issue has rarely been addressed concerning hyperbranched polymers. For comparison, we also synthesized a batch of HPAMAM-2 with M_n of 11 500 and PDI of 1.88 (quite close to the PDI of the above HPAMAM, 1.86). It was also further modified with PHE to obtain HPAMAM-2-PHE45. As shown in the Supporting Information (Figure S2), after 3:1 weight ratio, the HPAMAM-2/DNA complex sizes were >400 nm, whereas the mean sizes of HPAMAM-2-PHE45/DNA were ∼200 nm at around 25:1 to 30:1 weight ratios (ratios chosen by gel retardation assay; see Figure S1 of the Supporting Information) and increase with increasing weight ratio to reach ∼400 nm at 50:1 weight ratio. The surface ζ potentials of the complexes of pDNA with HPAMAM-2 increased from −3 to 59 as the mass ratio increased from 1:1 to 5:1. The ζ potentials of the HPAMAM-2-PHE/DNA complexes dramatically varied within the range of −3 to 55 mV with the increase in the mass ratio from 25:1 to 40:1.

For the larger HPAMAM-2, the PHE modification also had a significant impact on the gene transfection efficiency. The HAPAMAM-2-PHE45/DNA complexes at 45:1 (w/w) ratio exhibited much higher transfection efficiencies than HPAMAM-2/DNA complexes as well as the PEI control (Figure 9). Although the HPAMAM-2 was a little more toxic than PEI (25 kD), after being modified by PHE, the cytotoxicity of HAPAM-AM-2-PHE45 became the minimum. (See Figure S4 in the Supporting Information.)

From another point of view, we can find that HPAMAM with M_n of 5510 showed better performance than HPAMAM-2 (M_n = 11 500) in terms of gene transfection and cytotoxicity. The differences of physicochemical properties, gene transfection activity, and cytotoxicity between HPAMAM and HPAMAM-2

are likely attributed to their different molecular weights. Nevertheless, more studies are needed in the future to actually understand the details. We are also investigating other amino acid and peptide modifications to see if further improvements are possible, which will be reported later.

5. Conclusions

We demonstrated that PHE-modified hyperbranched PAMAMs have the potential to be safe and highly effective gene carriers. It has the advantage of low cytotoxicity and also high gene transfection efficiency in SMMC-7721 and COS-7 cells if compared with PEI.

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Supporting Information Available. Gel retardation assay, average size and ζ potentials for HPAMAM-2/DNA and HPAMAM-2-PHE45/DNA complexes, cytotoxicity of HPAMAM-2/DNA and HPAMAM-2-PHE45/DNA complexes evaluated in SMMC-7721 cell line. This information is available free of charge via the Internet at http://pubs.acs.org/.

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