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Evaluation of Hyperbranched Poly(amino ester)s of Amine Constitutions Similar to Polyethylenimine for DNA Delivery

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New hyperbranched poly(amino ester)s were synthesized via $A_3 + 2BB'B''$ approach, represented by the Michael addition polymerization of trimethylol-propane triacrylate (TMPTA) (A_3-type monomers) with a double molar 1-(2-aminoethyl)piperazine (AEPZ) (BB′B″-type monomer) performed in chloroform at ambient temperature. The results obtained by in situ monitoring the polymerization using NMR and MS indicated that hyperbranched poly(TMPTA1-AEPZ2) was formed via a $A(B'B'')_2$ intermediate, and the B″ (the formed 2° amine) was kept intact in the reaction. Therefore, poly(TMPTA1-AEPZ2) contained secondary and tertiary amines in the core and primary amines in the periphery similar to polyethylenimine (PEI). The chemistry of protonated poly(TMPTA1-AEPZ2) was further confirmed by ^{13}C NMR, and the molecular weight, the radius of gyration (R_g), and the hydrodynamic radius (R_h) were determined using GPC, small-angle X-ray scattering (SAXS), and laser dynamic light scattering (LDLS), respectively. The ratio of R_g/R_h of ca. 1.1 verified the hyperbranched structure. Protonated hyperbranched poly(TMPTA1-AEPZ2) is degradable and less cytotoxic as compared with PEI (25 K). Gel electrophoresis reflected that stable complexes could be formed from protonated hyperbranched poly(TMPTA1-AEPZ2) and DNA, and the size and ξ-potential of the complexes were characterized. Remarkably, protonated hyperbranched poly(TMPTA1-AEPZ2) showed transfection efficiency comparable to PEI (25 k) for in vitro DNA delivery.

Introduction

Lack of safe and efficient vectors for gene delivery is a main hurdle to the success of gene therapy. Nonviral vectors have attracted much attention due to immunogenic problems caused by viral vectors.[1−12] Different kinds of polymers have been investigated for preparing nonviral vectors; however, polyethylenimine (PEI) still is one of the best polymers for DNA delivery.[1−10] The high gene transfection efficiency of PEI is attributed to its proton sponge effect making gene vectors escape from endosome easily; and the simultaneous existence of primary, secondary, and tertiary amines in PEI contributes to the proton sponge effect.[3] However, PEI shows significant levels of cytotoxicity either in vitro or in vivo, and its long-term safety is problematic as well due to its nonbiodegradability.[3c] So biodegradable polymers of amine constitutions similar to PEI are desirable for preparing safe and efficient nonviral vectors for gene delivery.

However, no biodegradable polymers with amine constitutions similar to PEI have hitherto been reported.[5−11] Hyperbranched PEI is prepared via cationic polymerization of aziridine, a kind of AB_2 monomer, and a classic AB_2 approach to hyperbranched polymers[13−15] has also been applied to prepare biodegradable hyperbranched poly(amino ester)s containing tertiary amines in the core and primary amines in the periphery but without secondary amines.[5a] On the other hand, oligomer PEIs of lower toxicity were combined together through biodegradable units.[16−19] Oligo-PEIs can be classified as multifunctional monomers with equal reactivity, therefore, reproducibility was poor in those approaches linking oligo-PEIs directly due to the fact that gelation occurred easily as indicated by Flory's gelation theory.[20] Furthermore, the combined oligo-PEIs are not really biodegradable with respect to oligo-PEI fragments although they have amine constitutions similar to PEI.

Polymerizations of multifunctional monomers of suitable unequal reactivity have been demonstrated to be feasible for preparing hypebranched polymers. In addition to the merits of commercially available monomers and practical one-pot processes, those approaches can also provide unique hyperbranched polymers.[21−28] On the basis of the Michael addition polymerization of tirfunctional amines with diacrylates or diacylamides, we have obtained linear poly(amino ester)s or poly(amino amide)s containing secondary and tertiary amines in the backbones;[11,29,30] also hyperbranched poly(amino ester)s with tertiary amine in the core and tunable primary, secondary and tertiary amines in the periphery were

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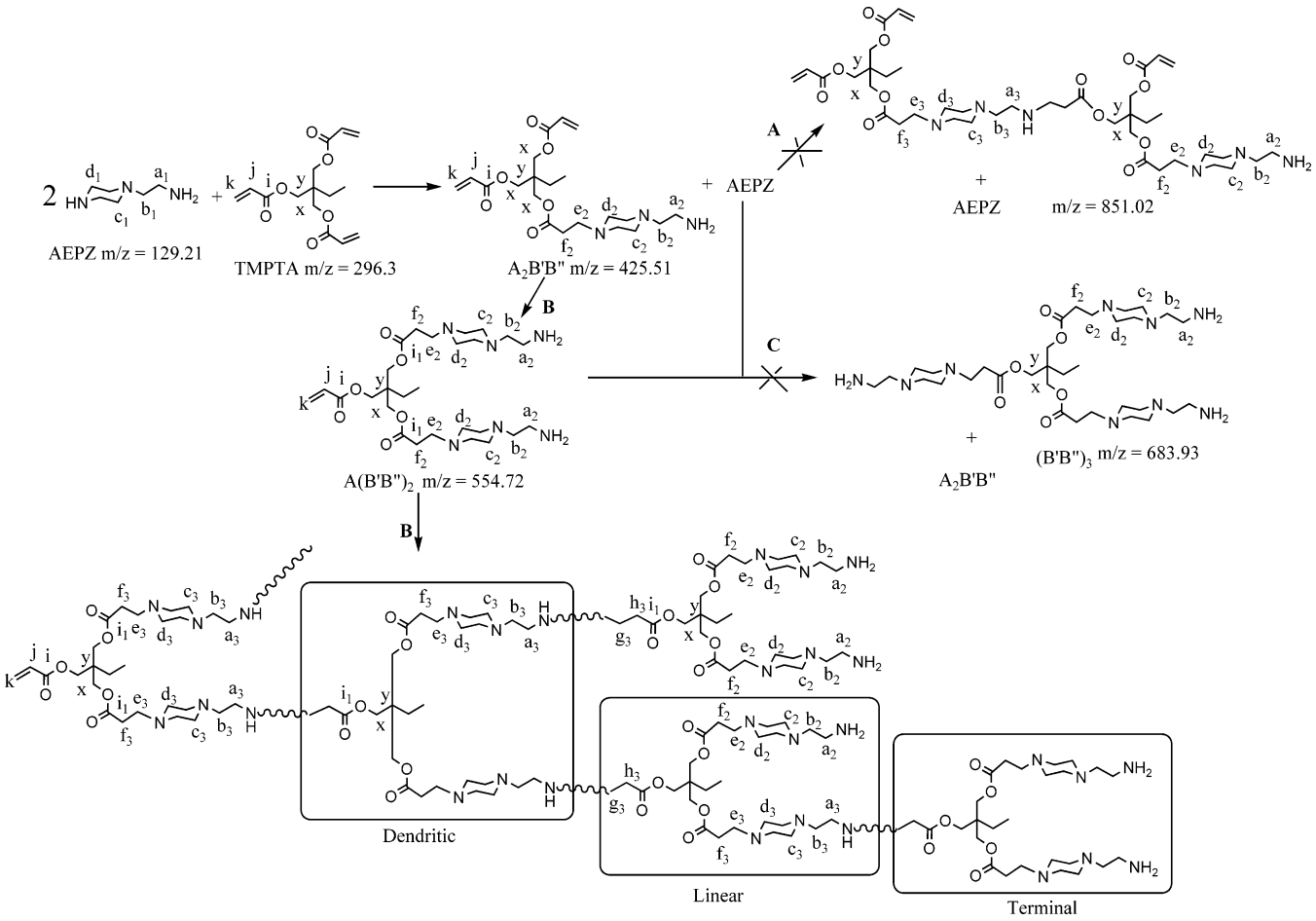
Scheme 1. Possible Reaction Routes in the Michael Addition Polymerization of TMPTA+ 2AEPZ



produced via a novel $2A_2 + BB'B''$ approach.[28] Here we exploited a novel $A_3 + 2BB'B''$ approach to hyperbranched poly(amino ester)s, where A_3 is a triacrylate, BB′(B″) is a trifunctional amine, and B, B′, and B″ represent the 2° amine (original), 1° amine, and 2° amine (formed), respectively. As described by route B in Scheme 1, hyperbranched poly(amino ester)s are expected to be formed via $A(B'B'')_2$ intermediate, which is formed through the reaction of the A_3 with the double molar B group of the highest reactivity. The B″, the 2° amine (formed), should be kept intact in the reaction due to the lowest reactivity; hence, novel poly(amino ester)s produced should contain primary, secondary, and tertiary amines similar to PEI. As a demonstration, this kind of hyperbranched poly(amino ester)s was prepared via the Michael addition polymerization of a triacrylate, trimethylolpropane triacrylate (TMPTA) (A_3-type monomer), with a double molar AEPZ (BB′B″-type-monomer). The polymerization mechanism was investigated. The chemistry and hyperbranched structure, the degradability and cytotoxicity, DNA condensation capability, and the transfection efficiency for DNA delivery in vitro of the poly(amino ester) obtained were characterized and evaluated.

Experimental Section

Characterization. ^{1}H NMR and ^{13}C NMR studies were performed on a Bruker DRX-400 spectrometer. Gel permeation chromatography (GPC) was carried out on a Waters 2690 apparatus with a column (Waters Ultrahydrogel 500 and 250) and a Waters 410 refractive index detector using 0.5 M acetic acid/0.5 M sodium acetate as the eluent at a flow rate of 0.5 mL/min. The molecular weights were calibrated against poly(ethylene oxide) standards or measured using a miniDAWN light scattering detector (Wyatt Technology). MS was conducted on a Finnigan TSQ 7000. The spray voltage was 4.5 kV, and the capillary temperature was set at 50 °C.

A Brookhaven BI-9000AT Digital Autocorrelator was used for dynamic light scattering measurements. The scattering angle was fixed at 90° for measuring the hydrodynamic radius (R_h). R_h and polydispersity (PDI), i.e., $\langle\mu_2\rangle/\Gamma^2$, were obtained using a cumulant analysis. Small-angle X-ray scattering (SAXS) was performed on a SAXS system (Bruker AXS Inc. NanoStar SAXS) with a pinhole collimation configuration and a Cu Kα ($\lambda = 1.5418$ Å) radiation source. The SAXS signal was detected using a two-dimensional multi-wire proportional HI-STAR area detector corrected for dark current, empty beam, solution cell, and solvent contributions. Samples were scanned at a voltage of 40 kV and a current of 35 mA. Scattering intensity $I(q)$ and the corresponding wave vector q were collected for analysis, where $q = 4\pi \sin\theta/\lambda$, with θ and λ being the scattering angle and the wavelength of X-ray, respectively.

Materials and Reagents. 1-(2-Aminoethyl)piperazine (AEPZ, 99%), trimethylol-propane triacrylate (TMPTA, 88%), deuterium chloroform, deuterium oxide, and MTT (3-(4,5-dimethyl-thiazol-2-yl)-2,5-diphenyl tetrazolium bromide) were from Aldrich (Milwaukee, WI). Chloroform and acetone were from Tedia. TMPTA was purified by passing a silica gel column using a mixture of hexane and diethyl ether (volume ratio = 2:1) as the eluent. The other reagents were used without further purification. Plasmid DNA (pCMV−Luc) was obtained from Elim Biopharmaceuticals, California.

In Situ Monitoring Polymerization Processes. Typically, TMPTA was added into a solution of a double molar AEPZ in deuterium chloroform in a NMR tube. The monomer concentrations were around 20% (w/v) and the polymerizations were performed at ambient temperature. ^{13}C NMR spectra were recorded using a power-gated decoupling program (PD). A total of 200 time scans taking around 10 min was enough to get a good ^{13}C NMR spectrum.

Synthesis of Hyperbranched Poly(TMPTA1-AEPZ2). In a typical process, 5.4 mmol of TMPTA was added dropwise to a solution of 10.8 mmol of AEPZ in 20 mL of chloroform under stirring. After the polymerization was conducted under ambient temperature for 8 days, the solution was precipitated into 400 mL of acetone containing 5 mL of hydrochloric acid (10 M). The polymer was collected and washed with acetone followed by drying in a vacuum at 50 °C for 5 days. A white powder (3.3 g; yield, 78.9%) was obtained. For MS (ESI), samples were drawn from the reaction mixture at certain periods followed by dilution to 1×10^{-6} g/mL using chloroform to avoid further reaction.

Cytotoxicity Assay of Poly(amino ester). Cos7 and 293 cells were cultured in DMEM supplemented with 10% FCS at 37 °C, 10% CO_2, and 95% relative humidity. For the cell viability assay, polymer solutions were prepared in serum supplemented tissue culture medium. pH and osmolarity of the preparations were routinely measured and adjusted to pH 7.4 and 280−320 mosm/kg. The cells (10 000 cells/well) were seeded into 96-well microtiter plates (Nunc, Wiesbaden, Germany). After overnight incubation, the culture medium was replaced with 100 μL serial dilutions of the polymers, and the cells were incubated for another 12 h. Then the medium with polymer extraction was aspirated and replaced by 100 μL of DMEM without serum to minimize the change of aggregate formation between the charged sites of proteins and polymer before adding MTT assays. A total of 20 μL of sterile filtered MTT (3-(4,5-dimethyl-thiazol-2-yl)-2,5-diphenyl tetrazolium bromide) (5 mg/mL) stock solution in phosphate buffered saline (PBS) was added to each well. After 4 h, unreacted dye was removed by aspiration. The formazan crystals were dissolved in 100 μL/well DMSO (BDH laboratory Supplies, England) and measured spectrophotometrically in an ELISA reader (model 550, Bio-Rad) at a wavelength of 570 nm. The spectrophotometer was calibrated to zero absorbance using culture medium without cells. The relative cell growth (%) related to control cells containing cell culture medium without polymer was calculated by $[A]_{test}/[A]_{control} \times 100\%$. All of the tests were performed in triplicate.

Formation and Analysis of DNA/Poly(amino ester) Complexes. Plasmid DNA (pCMV−Luc) was diluted to the chosen concentration (usually 0.5−2.0 μg/μL) in 5% glucose under vortexing. Various amounts of 0.1 M solution of poly(TMPTA1-AEPZ2) in 5% glucose were added slowly to the DNA solutions. The amount of poly(TMPTA1-AEPZ2) added was calculated based on chosen weight ratios of poly(TMPTA1-AEPZ2) to DNA. After the solution was incubated at ambient temperature for 30 min under gentle vortexing, the formed poly(TMPTA1-AEPZ2)/DNA complexes were mixed with a loading buffer and loaded onto a 1% agarose gel containing ethidium bromide. Gel electrophoresis was run at room temperature in HEPES buffer (20 mM, pH = 7.2) at 80 V for 60 min. DNA bands were visualized by an UV (254 nm) illuminator. The particle sizes and ξ-potentials of the complexes of 20 μg/mL DNA and 10−1200 μg/mL polymer in HEPES buffer (20 mM, pH = 7.2) were measured at 25 °C using a ZetaPALS dynamic light scattering detector (Brookhaven Instruments Corporation, Holtsville, NY, 15mW laser, incident beam = 676 nm).

DNA Delivery in Vitro. The in vitro transfection efficiency of the poly(amino ester) was evaluated in Cos7 and HEK 293 cells using the complexes formed with poly(TMPTA1-AEPZ2) and DNA (pCMV−Luc). Cells were seeded 24 h prior to transfection into 24-well plates (Becton-Dickinson, Lincoln Park, N.J) at a density of 5×10^4 per well with 0.5 mL of complete medium. At the time of transfection, 100 μL of polymer/DNA complexes with various w/w (or N/P) at a DNA dose of 2 μg/well was dropped into each well and incubated with the cells for 4 h at 37 °C. The medium was replaced with 0.5 mL of fresh complete medium and cells were further incubated for 48 h. After the medium was drawn out and the well was washed with 0.3 mL 1 × PBS, cells were permeabilized with 200 μL of 1 × cell lysis buffer (Promega Co., Wis.). After being frozen at −78 °C for 20 min followed by returning to room temperature for two cycles, the complexes were collected. After centrifugation (15 000/5 min at 4 °C), the samples were ready for testing. The luciferase activity in cell extracts was measured using a luciferase assay Kit (Promega Co., Madison, Wis.) on a single-well luminometer (Berthold Lumat LB 9507, Germany) for 10 s. The light units (LU) were normalized against protein concentration in the cell extracts, which was measured using a protein assay kit (Bio-Rad Labs, Hercules, California). All of the transfection tests were performed in quadruplicate.

Results and Discussion

Preparation of Hyperbranched Poly(amino ester)s. The polymerization of TMPTA + 2AEPZ was performed in chloroform at ambient temperature. ^{13}C NMR as shown in Figure 1c reflects that almost all of the vinyl groups in TMPTA were consumed in ca. 197 h, but no gelation occurred throughout the reaction. In general, this could be attributed to the different reactivities of the three types of amines in AEPZ.[11,28−30] However, further investigation was necessary to get a detailed understanding of the mechanism of polymerization. As described in Scheme 1, there are three

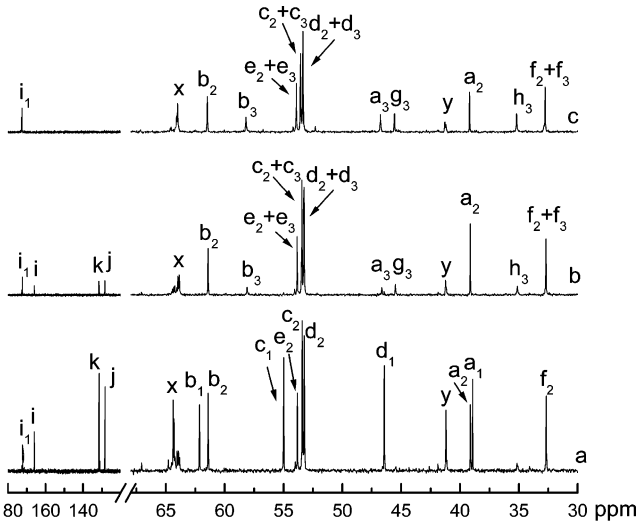


Figure 1. Comparison of ^{13}C NMR spectra recorded in situ for the polymerization of TMPTA + 2AEPZ with a monomer concentration of 20% (w/v) in $CDCl_3$ at ambient temperature for (a) 0.40 h; (b) 11.8 h; (c) 197.0 h.

possible reaction routes to the intermediates of the polymerization of TMPTA + 2AEPZ. First AEPZ reacts with an equimolar TMPTA forming TMPTA−AEPZ ($A_2B'B''$ intermediate). Then the self-polymerization of the $A_2B'B''$ intermediate may occur with the AEPZ monomer being left in the route A, or TMPTA−2AEPZ ($A(B'B'')_2$ intermediate) is formed through the reaction of AEPZ and $A_2B'B''$ intermediate in the route B, or the $A(B'B'')_2$ intermediate reacts with AEPZ provides TMPTA−3AEPZ ($(B'B'')_3$ intermediate) with coexistence of the $A_2B'B''$ intermediate in the route C.

To identify the reaction route, the polymerization was monitored in situ using ^{13}C NMR and MS (ESI). The results of ^{13}C NMR and MS (ESI) are shown in Figures 1 and 2, respectively. The ascription of the peaks in the ^{13}C NMR was based on our previous results.[28−30] Figure 1a indicates that the most reactive 2° amine (original) reacted with TMPTA forming the $A_2B'B''$ or $A(B'B'')_2$ intermediate as indicated by the peaks such as b_2 at ca. 61.4 ppm at the beginning of the reaction. When the polymerization was performed for ca. 11.8 h, Figure 1b shows that all of the 2° amines (original) in AEPZ were consumed as reflected by the total disappearance the peaks such as b_1 at 62.1 ppm, meanwhile, the appearance of the relatively weak peaks such as b_3 at 58.1 ppm indicated that only a little amount of the 2° amines (formed) were formed. Therefore, no reaction as described by the route A occurred. This is reasonable due to the lower reactivity of the 1° amine as compared with the 2° amine (original).

It is difficult to differentiate $A_2B'B''$, $A(B'B'')_2$, and $(B'B'')_3$ intermediates using NMR, however, MS (ESI) is a useful tool for this analysis. A model compound $(B'B'')_3$ was prepared via TMPTA + 4AEPZ, and a peak of the triply charged $(B'B'')_3$ at $m/z = 228.9$ appears in the MS (ESI) of the reaction product as shown in Figure 2a. However, no such a peak appears in the MS (ESI) of the product of the polymerization of TMPTA + 2AEPZ after 11.8 h as depicted in Figure 2b indicating no formation of $(B'B'')_3$. Note that at this stage AEPZ was consumed as indicated by ^{13}C NMR in Figure 1b, therefore, $A_2B'B''$ could not be found as well. The peak of the $A_2B'B''$ intermediate at $m/z = 426.29$ in Figure 2b should be the fragment formed in the MS (ESI) experiment. Therefore, only the $A(B'B'')_2$ intermediate existed as the precursor of the polymer as described by the route B, supported by the appearance of a relatively intensive peak of doubly charged $A(B'B'')_2$ intermediate at $m/z = 278.35$ in Figure 2b. Probably the steric hindrance and inductive effects of the two attached AEPZ groups in the $A(B'B'')_2$ intermediate reduce the reactivity of the remained vinyl groups, so route C was hindered.

Figure 1c shows that almost all of the peaks of vinyl groups disappeared after 197.0 h indicating that all of the $A(B'B'')_2$ intermediates were converted to hyperbranched poly (TMPTA1−AEPZ2); meanwhile, the 2° amine (formed) was kept intact in the reaction as illustrated by no appearance of the peaks adjacent to the tertiary amine from the 2° amine (formed).[29] Hence, the hyperbranched poly(TMPTA1−AEPZ2) produced contains secondary and tertiary amines in the core and primary amines in the periphery similar to PEI as described in Scheme 1. Moreover, hyperbranched poly(TMPTA1−AEPZ2) should have a focal unit containing a vinyl group as indicated in Scheme 1. The almost complete disappearance of the vinyl group in Figure 1c implies that hyperbranched poly(TMPTA1−AEPZ2) of a high molecular weight was produced.

Structures of Hyperbranched Poly(TMPTA1−AEPZ2). The polymerization of TMPTA + 2AEPZ could be performed in chloroform at ambient temperature due to the reasonable reactivity of the 2° amine (original) and 1° amine. Pure solid neutral poly(TMPTA1−AEPZ2) became insoluble in the purification process, probably caused by the further intermolecular reaction between the secondary and/or primary amine with the carbonyl group. So only soluble protonated poly(TMPTA1−AEPZ2) was obtained due to the reduced reactivity of the protonated amines. Figure 3 is the ^{13}C NMR spectrum of the protonated hyperbanched poly(TMPTA1-AEPZ2). There is one type of carbon linked to the primary amine with the peak a_1 at 42.6 ppm, two types of carbons linked to the secondary amine with the peaks a_2 and g_2 at 43.5 and 40.3 ppm, and eight types of carbons linked to the tertiary amine with the peaks at 48.4 ppm ($d_1 + d_2$), 48.8 ppm ($c_1 + c_2$), 51.4 ppm (b_1), 51.9 ppm ($e_1 + e_2$), and 52.5 ppm (b_2). The molecular weight (M_w) of the protonated poly(TMPTA1−AEPZ2) measured using GPC was ca. 23 260 based on poly(ethylene oxide) standards and ca. 36 930 as determined by a laser light scattering detector with a PDI of 1.68.

The DB of hyperbranched poly(TMPTA1−AEPZ2) should be evaluated by taking the $A(B'B'')_2$ intermediate as a starting monomer similar to the case of the hyperbranched poly(amino ester)s obtained via the $2A_2 + BB'B''$ approach,[28] so the terminal, linear, and dendritic units are defined as in Scheme 1. Nevertheless, the structural difference among the linear and terminal/dendritic units could not be distinguished in either ^1H or ^{13}C NMR, so the DB according to this definition was difficult to measure.

The ratio of the hydrodynamic radius (R_h) to the radius of gyration (R_g) of the polymer can provide information on

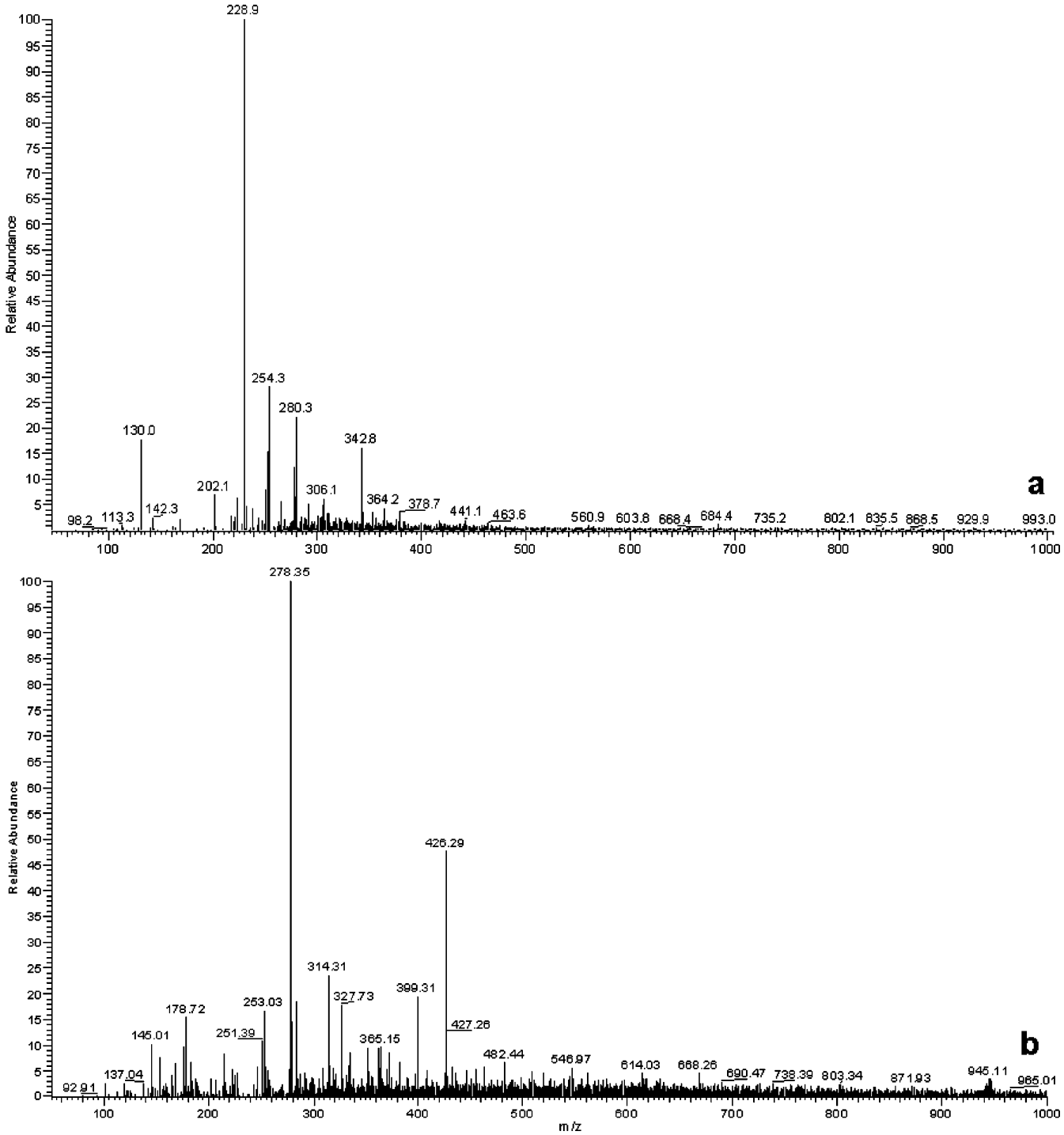


Figure 2. Mass spectrum of (a) the product of the reaction of TMPTA + 4AEPZ in chloroform at ambient temperature for 24 h and (b) the sample drawn from the polymerization solution of TMPTA + 2AEPZ in chloroform at ambient temperature for 11.8 h. The samples were diluted to 1×10^{-6} g/mL in chloroform for MS (ESI).

hyperbranched structures.[28,31−34] An aqueous solution of protonated poly(TMPTA1−AEPZ2) was prepared for the measurement of R_h and R_g. The R_h of poly(TMPTA1−AEPZ2) was measured to be 4.6 nm with a PDI of 0.238 using a laser dynamic light scattering (LDLS), and R_g was determined to be 5.1 nm using a SAXS.[31,32] The ratio of R_g/R_h for protonated poly(TMPTA1−AEPZ2) was 1.1 indicating that the spatial structure was similar to those obtained from AB_2-type monomers,[33,34] which coincided with the mechanism of polymerization as described in Scheme 1.

Degradability and Cytotoxicity. Similar to other kinds of poly(amino ester)s,[5,11] poly(TMPTA1−AEPZ2) is degradable through the hydrolysis of the ester group. The hydrolysis process of poly(TMPTA1−AEPZ2) in aqueous solution was monitored using ^{1}H NMR. Upon hydrolysis of the ester group, the peak attributed to the proton attached to the α carbon in the trimethylolpropane shifted from ca. 4.2 to ca.

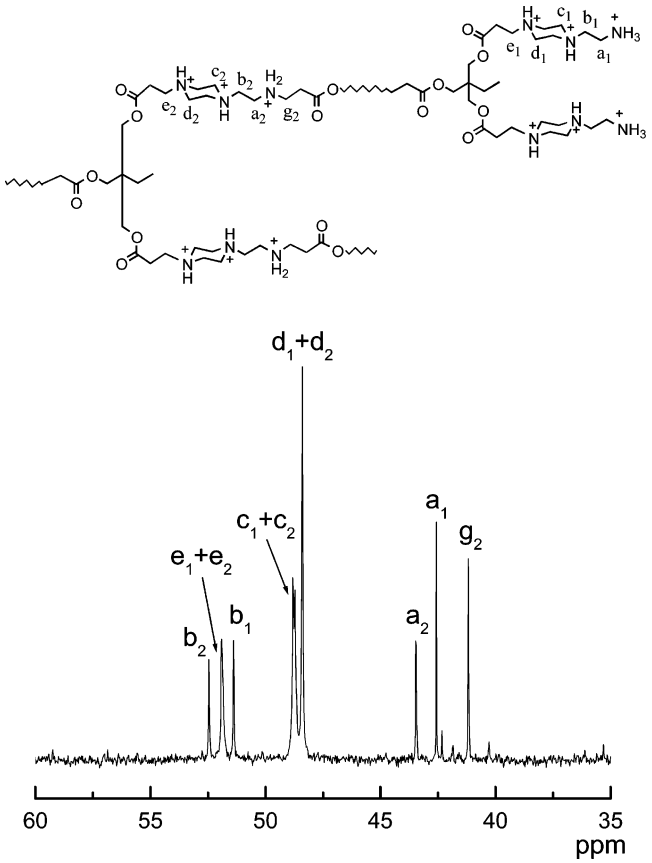


Figure 3. Enlarged ^{13}C NMR spectrum of protonated poly(TMPTA1−AEPZ2) in D_2O.

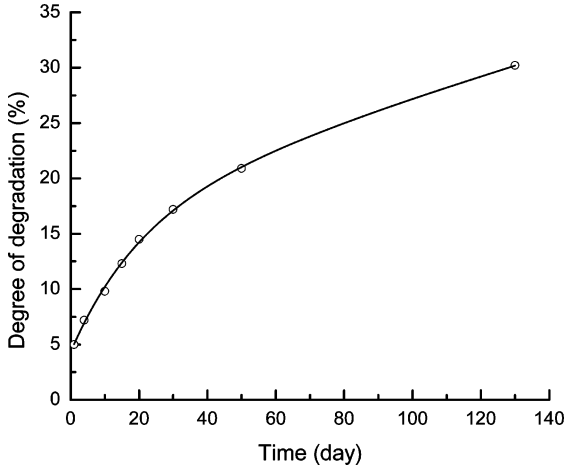


Figure 4. Hydrolysis profile of protonated poly(TMPTA1−AEPZ2) in aqueous solution.

3.5 ppm, but the peak attributed to the proton attached to the methyl group in the trimethylolpropane was kept unchanged at 0.9 ppm during the hydrolysis. Therefore, the degree of hydrolysis could be monitored by the change in the ratio of the integral intensities of the two peaks, $I_{4.2}/I_{0.9}$. As shown in Figure 4, around 30% of the ester groups were broken after 130 days in an aqueous solution. The hydrolysis is slower as compared to the protonated linear poly(amino ester)s.[11] Probably the densely hyperbranched spatial structure protects the ester group from hydrolysis.

The cytotoxicity of the protonated poly(TMPTA1−AEPZ2) was evaluated in HEK 293 cells. As depicted in Figure 4, the cell viability was still up to ca. 70% when the concentration of poly(TMPTA1−AEPZ2) was 500 μg/mL. In comparison, PEI (25 K) showed significant toxicity reflected by the fact that less than 20% of the cell was viable when the concentration of PEI (25 K) was higher than 40 μg/mL. A similar result was also obtained when a cytotoxicity assay of the polymer was performed in Cos7 cells as shown in Figure 5. So, protonated poly(TMPTA1−AEPZ2) has an excellent low cytotoxicity profile making it promising as a biomaterial. The cytotoxicity of cationic polymers was probably caused by polymer aggregation on cell surfaces impairing the important membrane functions.[35] Also polymers may interfere with critical intracellular processes of cells, especially, primary amine was reported to disrupt PKC function through disturbing protein kinase activity.[36] Poly(TMPTA1−AEPZ2) contains an amount of primary amines, therefore, its low cytotoxicity should result from its degradability in cells.

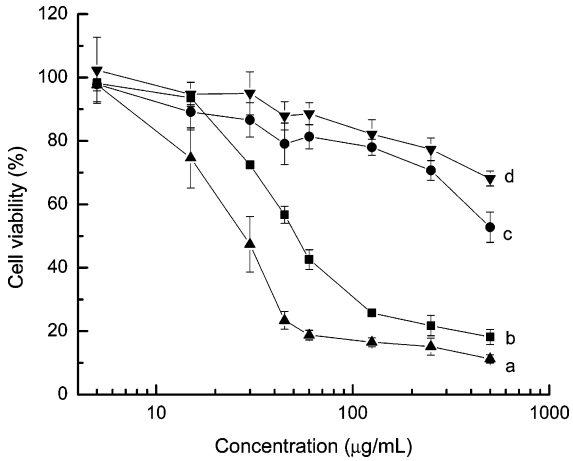


Figure 5. Comparison of the cytotoxicity of PEI (25 K) in (a) HEK 293 cells and (b) Cos7 cells and protonated poly(TMPTA1−AEPZ2) in (c) Cos7 cells and (d) HEK 293 cells. Values were presented as mean ± standard deviation ($n = 4$).

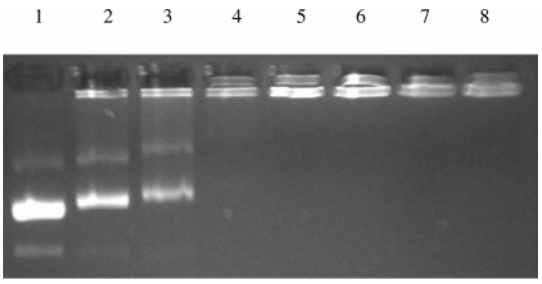


Figure 6. Agarose gel electrophoresis retardation of DNA (pCMV−Luc) by protonated hyperbranched poly(TMPTA1−AEPZ2). Lane numbers correspond to different DNA/polymer weight ratios as follows: (1) 1:0 (DNA only), (2) 1:0.6, (3) 1:0.8, (4) 1:1, (5) 1:1.5, (6) 1:2, (7) 1:3, and (8) 1:4.

DNA Condensation Capability. The protonated primary, secondary, and tertiary amines in poly(TMPTA1−AEPZ2) could interact with negatively charged DNA enabling formation of stable complexes. The results of agarose gel electrophoresis demonstrated that the migration of DNA was retarded completely when the weight ratio of poly(TMPTA1−AEPZ2)/DNA was higher than 1.5 as shown in Figure 6.

Figure 7 indicates that the biggest complex with a diameter of 687 nm was formed when the weight ratio of polymer/

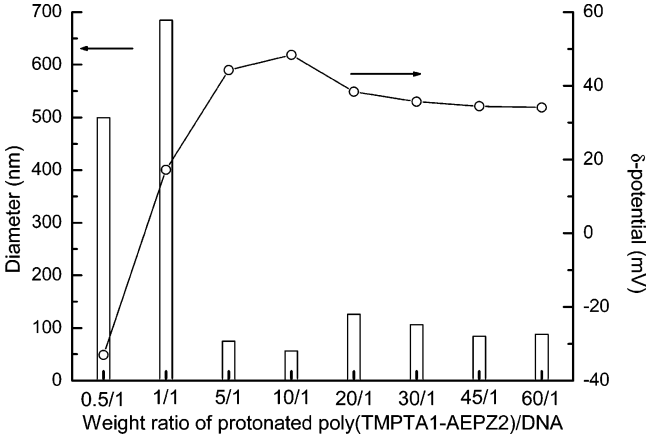


Figure 7. Effect of the weight ratio on the diameter and ξ-potential of the complex formed from 20 μg/mL of DNA (pCMV−Luc) and 10−1200 μg/mL of protonated hyperbranched poly(TMPTA1−AEPZ2) in HEPES buffer (20 mM, pH = 7.2) solution.

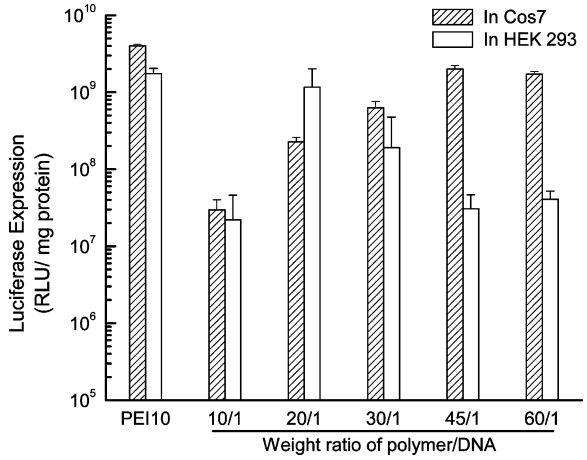


Figure 8. Transfection efficiency of the complexes of protonated poly(TMPTA1−AEPZ2)/DNA (pCMV−Luc) complexes in Cos7 and HEK 293 cells in comparison with that of PEI (25 kDa). The transfection efficiency of PEI (25 K) was obtained under an optimal N/P ratio of 10:1. Values were presented as mean ± standard deviation ($n = 4$).

DNA was 1/1, meanwhile a charge neutrality was achieved under this condition. So a neutral charge might result in aggregation of the complexes. When the polymer/DNA weight ratio was increased to be higher than 5:1, the positive charge of the complexes increased and leveled off at ca. 39 mV, and the diameter ranged from 50 to 150 nm as depicted in Figure 7.

DNA Transfection Efficiency In Vitro. Transfection efficiency of the complexes formed from protonated poly(TMPTA1−AEPZ2) and DNA was evaluated in Cos7 and HEK 293 cells, and the results are shown in Figure 8. The highest transfection efficiencies were obtained when the polymer/DNA weight ratios were ca. 45:1 in Cos7 cells and ca. 20:1 in HEK 293 cells. The values were around 50% and 70% of the control experiments employing PEI (25 k), respectively. It should be noted that this transfection efficiency was obtained in the absence of chloroquine, a commonly used weak base to enhance in vitro transfection through facilitating the release of DNA vectors from endosomes. The high transfection efficiency of poly(TMPTA1−AEPZ2) should be due to the proton sponge effect attributed to the simultaneous existence of primary, secondary, and tertiary amines similar to PEI, and the proton sponge effect facilitates the release of DNA from endosomes due to good buffer capability.[3] In addition, the biodegradability of poly(TMPTA1−AEPZ2) may also favor the release of DNA after the complexes entered cells, which also facilite DNA transfection.

Conclusions

A novel $A_3 + 2BB'B''$ approach, e.g., the Michael addition polymerization of trimethylol-propane triacrylate (TMPTA) (A_3-type monomer) with a double molar 1-(2-aminoethyl)piperazine (AEPZ) (BB′B″-type monomer), was set up to prepare new hyperbranched poly(amino ester). Hyperbranched poly(TMPTA1−AEPZ2) was formed via $A(B'B'')_2$ intermediate with the B″ (the 2° amine (formed)) being kept intact; therefore, hyperbranched poly(TMPTA1−AEPZ2) had an amine constitution similar to PEI, i.e., containing secondary and tertiary amines in the core and primary amines in the periphery. Water-soluble protonated poly(TMPTA1−AEPZ2) is degradable, showed low cytotoxicity, good DNA condensation capability, and transfection efficiency comparable to PEI (25 K) for DNA delivery in vitro. All of these properties make this novel kind of poly(amino ester) promising for preparing safe and efficient nonviral vectors for gene delivery.

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