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Synthesis and degradation of poly(beta-aminoester) with pendant primary amine

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Abstract

Three poly(beta-aminoester)s with pendant primary amines were synthesized by Michael addition of *N*-Boc-protected diamine to 1,4-butanediol diacrylate, followed by removal of *N*-Boc-protective group under anhydrous acidic conditions. The degradation rate of poly(beta-aminoester)s with pendant primary amines is dependant on their side-chain structures and pH value of incubation buffer. The degradation in basic environments is much faster than in acidic environments. The degradation of poly(beta-aminoester) with pendant primary amine involves intramolecular/intermolecular amidation and hydrolytic degradation. Under physiological conditions, the intramolecular/intermolecular amidation of poly(beta-aminoester) plays an important role in the polymer degradation. Polymers **1a**–**1c** show significant buffer capacity at pH 4–10. The cytotoxicity of polymer **1a** is much higher than that of polymers **1b** and **1c**.

Keywords: Poly(beta-aminoester); Pendant primary amine; Degradation

1. Introduction

Nonviral gene delivery systems have attracted more and more attention in the past two decades because of offering many advantages over viral systems, including ease of production, stability, low cytotoxicity and low immunogenicity, etc. [1–4]. Among the current nonviral methods, polycations are one of the most interesting groups of carriers widely investigated and applied in gene delivery [5–7]. A wide variety of natural and synthetic cationic polymers have been used to condense DNA and deliver DNA into cells, such as chitosan, gelatin, poly(L-lysine), poly(ethylenimine), poly(amidoamine) dendrimer. Most of the current polymeric gene carriers are nondegradable or nonhydrolytically degradable under physiological conditions. Recent interests in designing new polycationic gene carriers have in part focused on the introduction of hydrolysable linkage into the polymer main chains, such as ester [8–12], phosphoester [13–16] and phosphazene [17], to improve the biodegradability and decrease the toxicity of carriers. Poly(beta-aminoester)s are promising and efficient examples of hydrolysable polycations in nonviral gene delivery, which are often easy to be prepared via the Michael addition of primary amine or secondary amine to diacrylate. A library of poly(beta-aminoester)s has been synthesized and used as gene carriers. The chemical structures of poly(beta-aminoester)s, including main-chain structure and side-chain structure, greatly affect the transfection efficiencies [18–26]. In the previous work, hydroxyl groups, secondary and tertiary amino groups were introduced into poly(beta-aminoester)s side chains to improve the water solubility of polycations [18,19]. However, we have not found poly(beta-aminoester) with pendant primary amine synthesized and applied in gene delivery. Although there is no direct evidence to demonstrate that primary amino groups are necessary for highly efficient transfection mediated by cationic polymers, in fact, most of current efficient polycationic gene carries contain primary amines, such as poly(ethylenimine), dendrimer, etc. In this

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communication, we synthesized and characterized novel poly(beta-aminoester)s with pendant primary amines via Michael addition of *N*-Boc-protected diamine to butanediol diacrylate, followed by the deprotection of Boc-protective groups under acidic conditions. The significant advantages of introducing primary amines into polymer side chains involve improving the water solubility and enhancing the positive-charge density of polycations, which are important for highly efficient non-viral gene delivery. We also investigated the degradation of poly(beta-aminoester)s incubated in buffer at different pH values, buffer capacity and cytotoxicity to HeLa cells.

2. Experimental

2.1. Materials and methods

All solvents were of analytical grade, obtained from domestic suppliers and used directly. Di-*tert*-butyl dicarbonate was purchased from domestic suppliers and used without purification. 1,2-Diaminoethane and 1,6-diaminohexane were from domestic suppliers and distilled before used. 1,4-Diaminobutane was purchased from Aldrich and used without purification. 1,4-Butanediol diacrylate was purchased from Fluka. *N-tert*-Butoxycarbonyl-1,2-diaminoethane, *N-tert*-butoxycarbonyl-1,4-diaminobutane and *N-tert*-butoxycarbonyl-1,6-diaminohexane were synthesized from di-*tert*-butyl dicarbonate and respective diamines according to the literature method [27]. Deuterated phosphate buffer pH 7.4, citric acid–Na_2HPO_4 buffer pH 6.0 and citric acid–Na_2HPO_4 buffer pH 5.0 were prepared by dissolving the respective non-deuterated salts into D_2O.

FT-IR spectra were measured on Perkin–Elmer Spectrum one. ^{1}H NMR spectra were recorded on a Mercury VX-300 MHz spectrometer using tetramethylsilane (TMS) as an internal reference and $CDCl_3$ or D_2O as solvent. Gel permeation chromatographic (GPC) measurement was carried out by using a Waters-2690D HPLC equipped with Ultrahydrogel 120 and 250 columns. Sample was detected with a Wyatt multiangle light scattering detector and a Waters 2410 differential refractive index detector. Tris buffer (0.05 mol L^{-1}, pH 7.4) was used as the mobile phase at flow rate of 1.0 mL/min. The column temperature was 25 °C.

3-[4,5-Dimethylthiazol-2-yl]-2,5-diphenyltetrazoplium bromide (MTT) was purchased from Sigma. Dulbecco's Modified Eagle's Medium (DMEM), L-glutamine, and ampicillin were purchased from Gibco BRL (Grand Island, NY). Fetal bovine serum (FBS) was from Hyclone. HeLa cells were from Chinese Typical Culture Center (CTCC) (Wuhan University) and cultured in DMEM supplemented with 10% FBS, 4 mM L-glutamine, 100 μg/mL streptomycin, and 100 U/mL penicillin at 37 °C in 5% CO_2 atmosphere.

2.2. Synthesis of poly(beta-aminoester) with pendant N-Boc-protected amine (N-Boc-*1*)

N-Boc-**1a**: 1,4-butanediol diacrylate (0.99 g, 5 mmol) and *N-tert*-butoxycarbonyl-1,2-diaminoethane (0.8 g, 5 mmol) were mixed in a two-neck flask. The temperature of the oil bath was raised slowly to 120 °C. The mixture was stirred at this temperature for 12 h under N_2 atmosphere, then cooled to room temperature to form yellow solid. The crude product was dissolved in dichloromethane and precipitated in petroleum ether for three times, and dried under vacuum to obtain *N*-Boc-**1a** (0.92 g, yield 51.2%). IR: 3403, 2973, 2827, 1733, 1700, 1519, 1457, 1176 cm^{-1}; ^{1}H NMR ($CDCl_3$, ppm) δ: 1.28–1.60 (s, 9H, $3CH_3$), 1.60–1.84 (m, 4H, $2CH_2$), 2.30–2.52 (m, 4H, $2CH_2$), 2.52–2.68 (m, 2H, CH_2), 2.68–2.84 (m, 4H, $2CH_2$), 2.95–3.20 (m, 2H, CH_2), 4.0–4.24 (m, 4H, $2CH_2$).

N-Boc-**1b** was synthesized from 1,4-butanediol diacrylate and *N-tert*-butoxycarbonyl-1,4-diaminobutane according to the procedure described above, yield 47.7%. IR: 3423, 2989, 2852, 1732, 1700, 1651, 1520, 1453, 1278, 1172 cm^{-1}; ^{1}H NMR ($CDCl_3$, ppm) δ: 1.30–1.58 (m, 11H, $CH_2 + 3CH_3$), 1.58–1.84 (m, 6H, $3CH_2$), 2.28–2.50 (m, 6H, $3CH_2$), 2.62–2.82 (m, 4H, $2CH_2$), 2.96–3.18 (m, 2H, CH_2), 4.0–4.22 (m, 4H, $2CH_2$).

N-Boc-**1c** was similarly synthesized from 1,4-butanediol diacrylate and *N-tert*-butoxycarbonyl-1,6-diaminohexane, yield 45.5%. IR: 3381, 2932, 2858, 1733, 1700, 1521, 1458, 1173 cm^{-1}; ^{1}H NMR ($CDCl_3$, ppm) δ: 1.18–1.36 (m, 4H, $2CH_2$), 1.36–1.62 (s, 9H, $3CH_3$), 1.62–1.90 (m, 8H, $4CH_2$), 2.40–2.80 (m, 6H, $3CH_2$), 2.80–3.15 (m, 6H, $3CH_2$), 3.96–4.10 (m, 4H, $2CH_2$).

2.3. Synthesis of poly(beta-aminoester) with pendant primary amine (*1a–1c*)

Amine **1a**: a solution of *N*-Boc-**1a** (0.92 g) in 10 mL of dry ethyl acetate was added dropwise to ice-cooled 30 mL of ethyl acetate saturated with dry HCl gas with vigorous stirring. The mixture was stirred in ice-bath for further 30 min, and then concentrated in vacuum to yield the semi-solid product, which was dissolved in distilled water and lyophilized to obtain polymer **1a** as white solid (0.77 g, yield 91.4%). IR: 3433, 2965, 2836, 1730, 1407, 1192 cm^{-1}; ^{1}H NMR (D_2O, ppm) δ: 1.50–1.70 (m, 4H, $2CH_2$), 2.64–2.90 (m, 4H, $2CH_2$), 3.14–3.60 (m, 8H, $4CH_2$), 3.94–4.20 (m, 4H, $2CH_2$). $M_w = 1.01 \times 10^4$, $M_w/M_n = 1.26$.

Amine **1b** was similarly synthesized from *N*-Boc-**1b** and solution of saturated dry HCl in ethyl acetate, yield 87.8%. IR: 3427, 2943, 2836, 1729, 1631, 1409, 1192 cm^{-1}; ^{1}H NMR (D_2O, ppm) δ: 1.39–1.75 (s, 8H, $4CH_2$), 2.65–2.85 (m, 4H, $2CH_2$), 2.85–2.95 (m, 2H, $2CH_2$), 2.95–3.10 (m, 2H, CH_2), 3.15–3.38 (m, 4H, $2CH_2$), 4.12 (m, 4H, CH_2). $M_w = 8.34 \times 10^3$, $M_w/M_n = 1.20$.

Amine **1c** was similarly synthesized from Boc-**1c** and solution of saturated dry HCl in ethyl acetate, yield 84.8%. IR: 3421, 2955, 2826, 1732, 1409, 1172 cm^{-1}; ^{1}H NMR (D_2O, ppm) δ: 1.20–1.30 (m, 4H, $2CH_2$), 1.4–1.78 (m, 8H, $4CH_2$), 2.75–2.9 (m, 6H, $3CH_2$), 3.05–3.15 (m, 2H, $2CH_2$), 3.25–3.45 (m, 4H, $2CH_2$), 3.98–4.16 (m, 4H, $2CH_2$). $M_w = 6.28 \times 10^3$, $M_w/M_n = 1.40$.

2.4. Degradation of poly(beta-aminoester) with pendant primary amine (**1a–1c**)

The degradation of poly(beta-aminoester) with pendant primary amine was performed according to the following typical procedure. Polymer of 10 mg was dissolved in 0.5 mL of phosphate buffer (0.1 M, dissolved in D_2O, pH 7.4) in a NMR tube. The NMR tube was incubated in 37 °C water bath, and pulled out periodically to measure ^{1}H NMR spectra. The percentage of relative ester bonds in reference to original polymer was calculated by the following equation. P4.0 and P3.45 represent the integral of the peak at δ 4.0 and 3.45, respectively.

$$\text{Percentage of relative ester bond in reference to original polymer } (\%) = \text{P4.0}/(\text{P4.0} + \text{P3.45}) \times 100$$

The degradation of **1b** in buffer at pH 6.0 and 5.0 was carried out similarly except using citric acid–Na_2HPO_4 buffer (dissolved in D_2O, 0.1 M, pH = 6.0 or 5.0) as incubation media.

2.5. Buffer capacities of polymer **1a–1c**

First, **1a–1c** was separately dissolved in 10.0 mL of 0.15 mol L^{-1} NaCl. The concentration of primary amine hydrochloride in polymer solution is 5 mmol L^{-1}. Potentiometric titration was performed with a pH meter using 0.1 mol L^{-1} NaOH as titrant. When the pH value of polymer solution was brought to 10 with NaOH, the solution was subsequently titrated with 0.1 mol L^{-1} HCl.

2.6. In vitro cytotoxicity

Polymers **1a–1c** were separately dissolved in DMEM containing 4 mM glutamine, 10% FBS, and penicillin and streptomycin (100 U/mL), and sterile-filtered. The solution was diluted in complete DMEM to different concentrations ranging from 3.4 μg/mL to 1000 μg/mL. HeLa cells were seeded into a 96-well plate at a density of 5000 cells/well in 50 μL of complete DMEM. After the cells were cultured at 37 °C for 1 day in an atmosphere containing 5% CO_2, the polymer solutions at a different concentration (50 μL) were added to the wells containing cells and complete DMEM (50 μL). Cells were cultured in complete medium containing polymer for further 24 h at 37 °C, and then MTT solution (5 mg/mL, 25 μL) in PBS buffer (pH 7.4) was added to each well except that PBS buffer (25 μL) was added for background wells. The mixtures were incubated at 37 °C for 2 h, before 100 μL of SDS solution (20% w/v SDS in 50:50 v/v mixture of DMF and water, pH 4.7) was added to each well and maintained at 37 °C overnight to dissolve the formazan blue crystal. The absorbance of the solution was measured using microplate reader (Bio-Rad 550, Hercules, CA) at 570 nm. The percentage relative viability in reference to control wells containing complete DMEM without the added polymer was calculated by the following equation (A: absorbance at 570 nm, A_0 is the absorbance of the solution containing cells and complete DMEM without MTT and polymer):

$$\text{Relative cell viability}(\%) = 100 \times (A_{\text{test}} - A_0)/(A_{\text{control}} - A_0)$$

3. Results and discussion

3.1. Synthesis of poly(beta-aminoester) with pendant primary amine

Previously reported poly(beta-aminoester)s were synthesized via the polyaddition of amine to diacrylate. Non-functionalized poly(beta-aminoester)s were easily prepared by one-step Michael addition procedure. Some functional side chains, such as hydroxyl groups, can also be directly introduced by one-step Michael addition [18,19]. However, it is unsuitable for the direct introduction of pendant primary amines into the poly(beta-aminoester)s. In this work, we prepared poly(beta-aminoester)s with pendant primary amine via a two-step process, which is shown in Fig. 1. First, poly(beta-aminoester)s containing *N*-Boc amino side chains were synthesized via the polyaddition of *N*-Boc-protected diamine [27] to 1,4-butanediol diacrylate. Second, *N*-Boc-protective groups in the side chains of poly(beta-aminoester)s were removed by acidic cleavage of *N*-Boc linkage to yield hydrochloride of poly(beta-aminoester)s with pendant primary amine.

Michael addition of *N*-Boc-protected diamine to 1,4-butanediol diacrylate may be performed in solvents, such as methanol, chloroform, DMSO. However, the polymerization in the absence of solvent and at high temperature is much faster than that in solvent. High monomer concentration in the absence of solvent is also important to decrease the intramolecular cyclization reaction and yield high-molecular weight polymer. Additionally, molar ratio of monomers is the most important factor for controlling polymer molecular weight [20]. Theoretically, maximum molecular weight polymer should be obtained when molar ratio of amine to diacrylate is 1:1. Based on the above discussion, we performed the polymerization of stoichiometric equivalent *N*-Boc-protected diamine and butanediol diacrylate at 120 °C for 12 h to synthesize Boc-**1**. With the increase of reaction time, the reaction mixture became more and more viscous. The cooled crude product was dissolved in dichloromethane and precipitated in petroleum ether to remove low-molecular weight polymer. *N*-Boc-**1a**, *N*-Boc-**1b** and *N*-Boc-**1c** were prepared from butanediol diacrylate and respective *N*-Boc-protected diamines, which were characterized by IR and ^{1}H NMR.

N-Boc-protective group is stable in the process of polymerization, purification and storage of polymer, which is cleavable under acidic conditions. In order to avoid the possible hydrolytic degradation of poly(beta-aminoester) in aqueous hydrochloride, we choose a saturated solution of dry hydrochloride in ethyl acetate for the deprotection of *N*-Boc group. Comparing the FT-IR and ^{1}H NMR spectra of **1** and respective *N*-Boc-**1**, we can conclude that *N*-Boc-protective groups were successfully removed and hydrochloride of

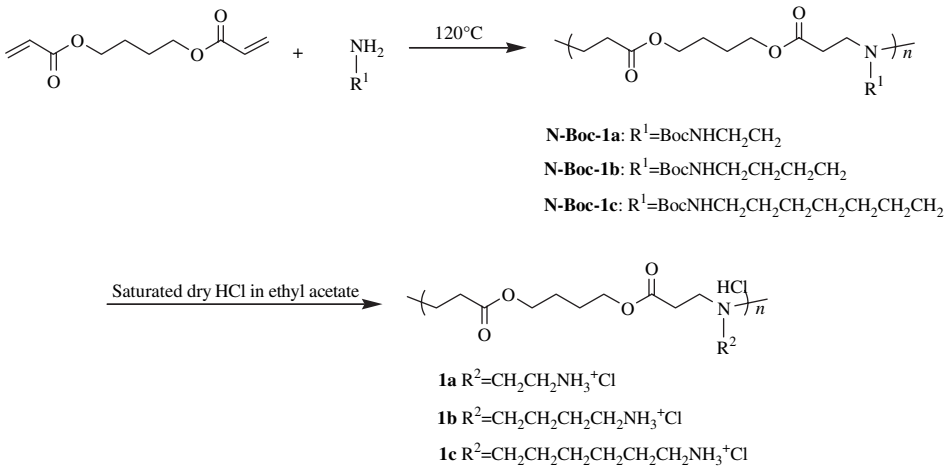

Fig. 1. Synthesis of poly(beta-aminoester)s with pendant primary amine **1a**–**1c**.

poly(beta-aminoester)s with pendant primary amine was obtained. Polymers **1a**–**1c** were analyzed by GPC–LS, using Tris–HCl buffer (pH 7.4) as eluent. The average molecular weight (M_w) of **1a**–**1c** is 6280, 8340 and 10 100, respectively. Molecular weight distributions were monomodal with polydispersity indices (PDIs) ranging from 1.20 to 1.40.

3.2. Degradation of poly(beta-aminoester) with pendant primary amine

Previous work has demonstrated that linear or hyperbranched poly(beta-aminoester) degrades in aqueous solution [25,28]. We here measured the degradation kinetics of poly(beta-aminoester)s with pendant primary amine using ^{1}H NMR at 37 °C in deuterated phosphate buffer (0.1 M, pH 7.4) or citric acid–Na_2HPO_4 buffer (0.1 M, pH 6.0 or 5.0). When the degradation of poly(beta-aminoester) occurred, the integral of signal at δ 4.00 gradually decreased and the integral of signal at δ 3.45 gradually increased. The content of ester degradation was calculated by comparing the integral of signal at δ 4.00 and the integral of signal at δ 3.45, attributed to the methylene protons adjacent to the ester (COOCH_2) and the hydroxyl group (HOCH_2), respectively. The degradation profiles of polymers **1a**–**1c** in phosphate buffer at pH 7.4 are shown in Fig. 2. The data demonstrated that the degradation rate of **1b** is the fastest among **1a**, **1b** and **1c**. The half-lives for ester degradation in **1a**–**1c** in phosphate buffer at pH 7.4 were approximately 144 h, 48 h and 72 h, respectively. The chemical structures of the main chains of **1a**–**1c** are same. This means that the degradation rate differences of **1a**–**1c** should be attributed to the differences of side chains. However, the relationship between the degradation rate and hydrophilicity of **1a**–**1c** is different from that of poly(beta-aminoester)s without pendant primary amine. For poly(beta-aminoester)s without pendant primary amine, more hydrophobic polymer degrades more slowly than hydrophilic polymer in aqueous solution. For poly(beta-aminoester)s with pendant primary amine **1a**–**1c**, the most hydrophilic polymer **1a** degrade most slowly. The results can be explained by the different degradation mechanism of poly(beta-aminoester)s with pendant primary amine and poly(beta-aminoester)s without pendant primary amine.

It has been speculated that the exact mechanism of poly(aminoester) degradation may include attack by both the free hydroxyl ion and intramolecular/intermolecular nucleophilic amine [10,11]. Because of the hindered reactivity of tertiary amine in poly(beta-aminoester)s without pendant primary amine, the nucleophilic tertiary amine is less important for the degradation in aqueous solution. As a result, the hydrophilicity of polymer controlled the degradation rate. In contrast, there are many free primary amine side chains in poly(beta-aminoester)s with pendant primary amine in phosphate buffer at pH 7.4, which play an important role for intramolecular/intermolecular nucleophilic attack to ester bond and formation of amide. The formation of amide during the polymer degradation was confirmed by the appearance of signal at δ 3.15 attributed to the methylene protons adjacent to the amide (CONHCH_2). The integral of signal at δ 3.15 increased with the increase of

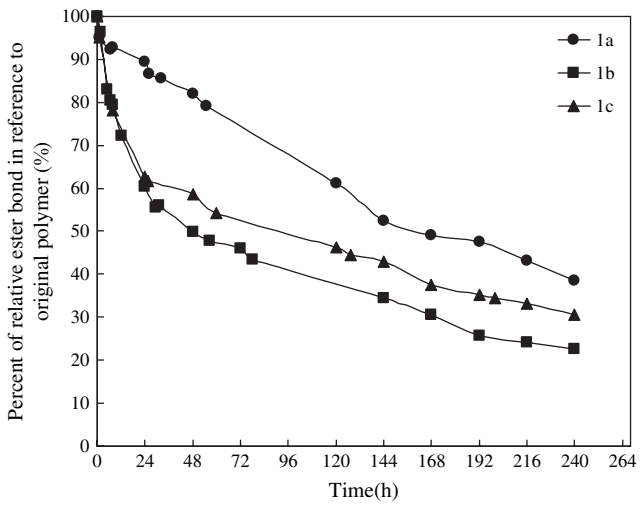

Fig. 2. The degradation profiles of polymers **1a** (●), **1b** (■) and **1c** (▲) at 37 °C in deuterated PBS buffer at pH = 7.4.

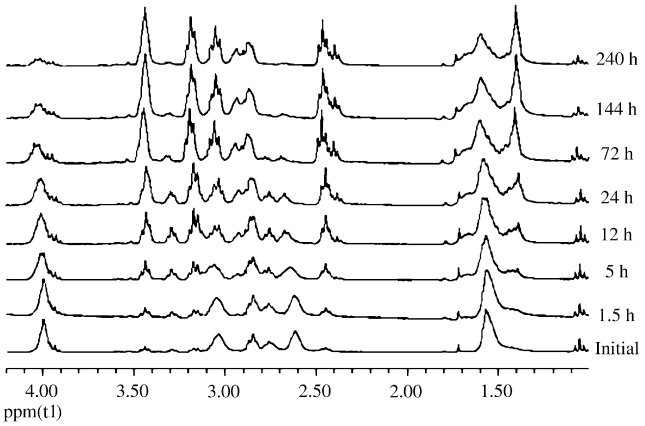

Fig. 3. The ^{1}H NMR spectra of **1b** degraded in deuterated phosphate buffer (0.1 M, pH 7.4) at different degradation time.

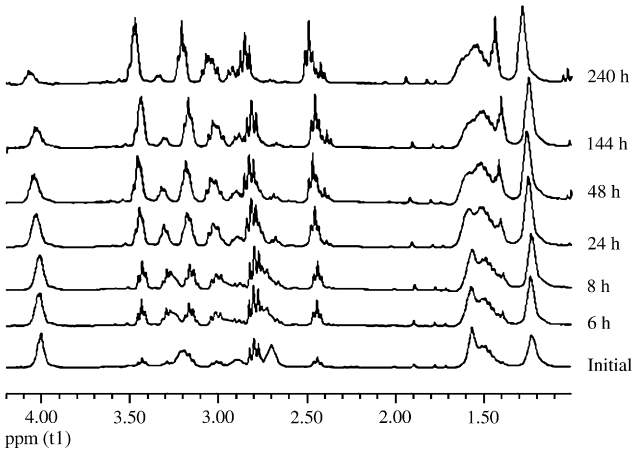

Fig. 5. The ^{1}H NMR spectra of **1c** degraded in deuterated phosphate buffer (0.1 M, pH 7.4) at different degradation time.

degradation time. The ^{1}H NMR spectra of polymer **1b** at different degradation time, shown in Fig. 3, indicated that the integral of signal at δ 3.15 at the early stage of degradation (before half-life) is almost equal to the integral of signal at δ 3.45. This means that the ester degradation at this stage is mainly contributed by intramolecular/intermolecular amidation, and the degradation rate is relatively fast. After the primary amines in the polymer were used-up, the polymer degradation is only contributed by hydrolysis, and the hydrolytic degradation rate is relatively slower than amidation. The possible degradation mechanism of **1b** was suggested and shown in Fig. 4. Similar results were observed in the degradation of **1c** in phosphate buffer at pH 7.4 (see Fig. 5). However, **1a** shows different degradation characteristics (see Fig. 6). Different from **1b** and **1c**, the intramolecular/intermolecular amidation in **1a** is very slow in the whole process of degradation. This implies that the intramolecular/intermolecular amidation in **1a** is much more difficult than that in **1b** and **1c**, and the degradation rate of **1a** is mainly controlled by the hydrolysis of ester. Consequently, the degradation rate of **1a** is much slower than that of **1b** and **1c**.

It is known that amine-containing polyesters degrade more rapidly in basic solution than in acidic solution. The degradation of **1b** in buffer at pH 7.4, 6.0 and 5.0 was carried out to evaluate the effect of buffer pH on the degradation rate of poly(beta-aminoester) with pendant primary amine. As shown in Fig. 7, the degradation rate of **1b** follows the order pH 7.4 > pH 6.0 > pH 5.0. The half-lives of esters in **1b** at pH 7.4, 6.0 and 5.0 were 48 h, 14 days and >65 days, respectively. It is easily understood that the protonation of pendant primary amine in **1b** in acidic solution inhibits the intramolecular/intermolecular nucleophilic attack of amine to ester and significantly decreases the degradation rate. The degradation rate of poly(beta-aminoester) with pendant primary amine is highly dependant on the solution pH value, namely, the degradation rate increases with the increase of solution pH value.

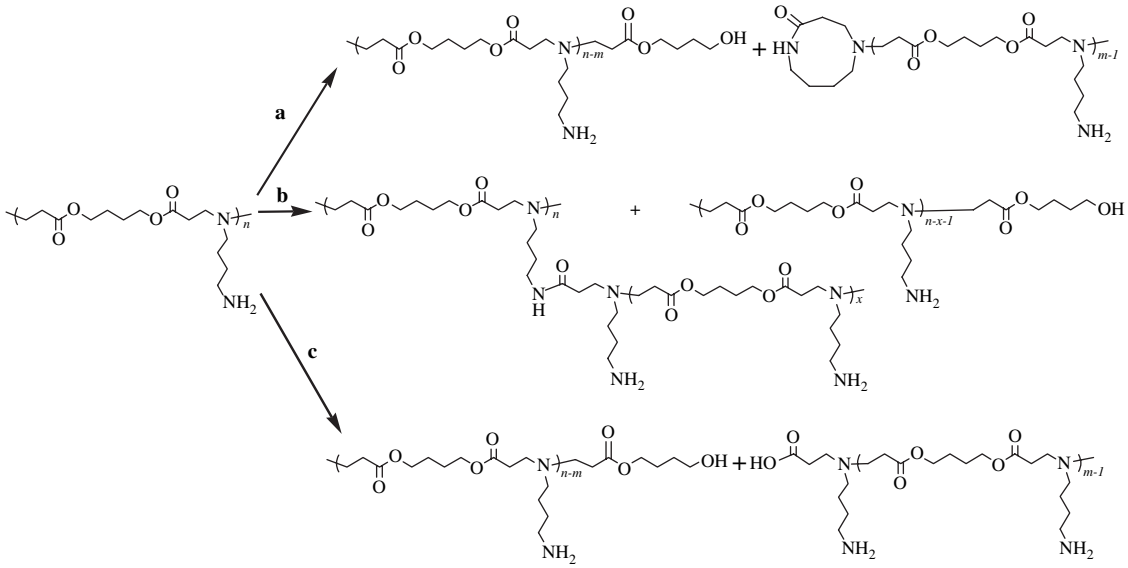

Fig. 4. The degradation route of **1b** in phosphate buffer at pH 7.4. Route a: degradation via intramolecular amidation; route b: degradation via intermolecular amidation; route c: hydrolysis of ester in polymer.

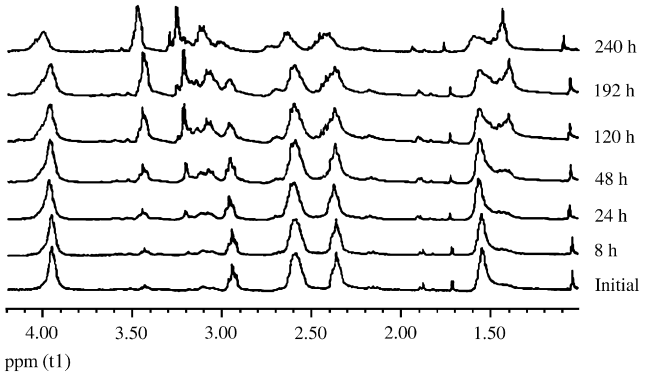

Fig. 6. The ^{1}H NMR spectra of **1a** degraded in deuterated phosphate buffer (0.1 M, pH 7.4) at different degradation time.

3.3. Buffer capacity of polymer *1a–1c*

According to the "proton sponge hypothesis" proposed by Behr et al., the buffer capacity of polycation at low pH (pH = 4–6) may play an important role in nonviral gene delivery, which facilitates endosomal escape of polycation/DNA complexes [29]. We first separately titrated the solution of polymers **1a–1c** in 0.15 mol L^{-1} NaCl with 0.1 mol L^{-1} NaOH to pH 10, and then titrated the solution with 0.1 mol L^{-1} to pH 3. The results of acid titration of polymers **1a–1c** are shown in Fig. 8. We can find that there are almost no differences among the buffer capacity of polymers **1a–1c** at higher pH (pH = 8–10), they all show high buffer capacity. However, at lower pH ranging from 4 to 6, which has been attributed to the endo/lysosomal compartment, the buffer capacity of polymer **1a** is relatively higher than polymers **1b** and **1c**. The effects of the buffer capacity of polymers **1a–1c** on the transfection efficiency are being evaluated by our group.

3.4. In vitro cytotoxicity of polymers *1a–1c*

The biocompatibility is an important factor for a cationic polymer in biomedical application. MTT assay was carried out to evaluate the cytotoxicity of polymers **1a–1c** in HeLa cells [30]. The results in Fig. 9 show that the cytotoxicity of polymers **1a–1c** depends on their side-chain structure. The IC_{50} of polymers **1a–1c** is 7.5, 85 and 130 μg/mL, respectively. This means that the cytotoxicity of polymer decreases with the increase of the length of the side chain. The biocompatibility of polymers **1b** and **1c** in HeLa cells is much higher than **1a**. Polycation would be complexed with DNA before applied in gene delivery. Generally, the polycation/DNA complexes show less toxicity than polymer alone.

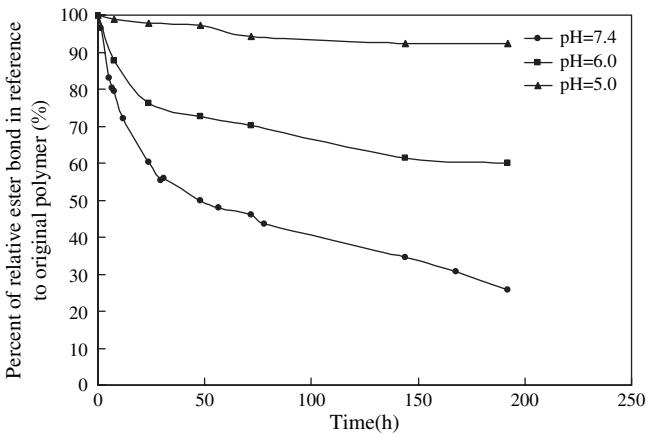

Fig. 7. The degradation profiles of **1b** at 37 °C in deuterated phosphate buffer pH 7.4 (●), citric acid–Na_2HPO_4 buffer pH 6.0 (■) and citric acid–Na_2HPO_4 buffer pH 5.0 (▲).

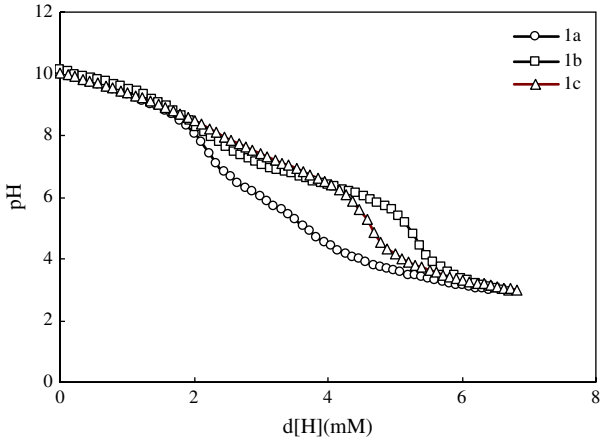

Fig. 8. The titration curve of polymers **1a–1c**. A solution of polymer (5 mM primary amine) in 0.15 mol L^{-1} NaCl was brought to pH 10 with 0.1 mol L^{-1} NaOH, and then subsequently titrated with 0.1 mol L^{-1} HCl.

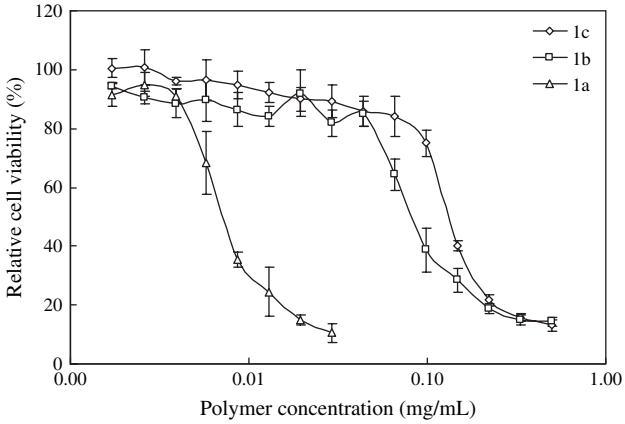

Fig. 9. In vitro cytotoxicity of polymers **1a–1c** in HeLa cells ($n = 4$).

4. Conclusion

In summary, novel poly(beta-aminoester)s with pendant primary amine were synthesized via Michael polyaddition of *N*-Boc-protected diamines to diacrylate, followed by the deprotection of *N*-Boc-protective group under anhydrous acidic conditions. The degradation rate of this kind of poly(beta-aminoester)s under physiological conditions is controlled by their side-chain structures. The intramolecular/intermolecular amidation in poly(beta-aminoester) plays an important role in the degradation. Solution pH value is another important factor to affect the degradation rate. The degradation of

poly(aminoester)s with pendant primary amine in basic environments is much faster than in acidic environments. Polymers **1a–1c** show significant buffer capacity at pH 4–10. The cytotoxicity of polymer **1a** is much higher than that of polymers **1b** and **1c**. The interaction of these degradable polycations with plasmid DNA and their application in nonviral gene delivery is in progress in our group.

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