A Self-Destroying Polycationic Polymer: Biodegradable Poly(4-hydroxy-L-proline ester)

Yong-beom Lim, Young Hun Choi, and Jong-sang Park*

Contribution from the Department of Chemistry, Seoul National University, Seoul 151-742, Korea

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Abstract: A self-destroying, biodegradable, and polycationic polyester, poly(*trans*-4-hydroxy-L-proline ester) (PHP ester), was synthesized, and the interaction of the polymer with polyanion DNA was investigated. Degradation of the polymer in aqueous solution was investigated by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) and by measuring the pH change as carboxylic acids are formed as products of the degradation of the polymer backbone ester bond. It was shown from MALDI-MS data that the polymer degraded to less than half of the intact polymer molecular weight in less than 2 h. But a slower degradation rate after initial rapid degradation (within 1 day) was apparent. A self-destroying mechanism at the initial stage is proposed. The polymer was gradually degraded to near completion in 3 months in an aqueous solution to monomer, hydroxyproline, a major constituent of collagen, which could easily be detected by using MALDI-MS. Although the polymer degraded very quickly in an aqueous solution, it formed stable PHP ester/DNA complexes by electrostatic interaction when the polymer was mixed with the polyanionic DNA solution. The condensation behavior of DNA with the polymer to form self-assembled PHP ester/DNA complexes was characterized by electrophoretic mobility shift assay, dynamic light scattering, ζ potential, and nuclease resistance assay. These results show that PHP ester forms a strong complex with DNA by means of electrostatic interaction. Transfection of β-galactosidase gene into mammalian cell using PHP ester/DNA complexes was successful, showing the possibility of using PHP ester as a biodegradable gene delivery carrier.

Introduction

Biodegradable polymers with hydrolyzable chemical bonds are gaining growing attention in biomedical, pharmaceutical, agricultural, and packaging uses.[1,2] The hydrolyzable chemical bonds include esters, amides, ortho esters, acetals, glycosides, and related groups. Polymers with these bonds are poly(amino acid)s,[3,4] poly(ester amide)s,[5] polydepsipeptides,[6,7] glycopeptides,[8] pseudopoly(amono acid)s,[9,10] and poly(ether urethane)s.[11]

One of the important criteria for the use of synthetic polymers in biomedical applications is that polymers should not only be biodegradable but also have pendant functional groups to which drugs or biologically active compounds can be attached covalently or noncovalently.[12] Poly(ϵ-caprolactone) bearing either ketone or hydroxyl pendant groups was synthesized.[13] Homopolymers and copolymers derived from L-glutamic acid[14] and L-aspartic acid[4] have side-chain carboxyl groups, which enabled covalent attachment of other functional groups, including drug molecules.[4] In comparison, poly(L-lactide-*co*-L-lysine)[7] and poly(serine ester)[15] have side-chain amine groups. Because side-chain carboxyl group-containing polymers become polyanionic and those with amine groups become polycationic, they can be used to attach the biologically active compounds via noncovalent an electrostatic interaction as well as a covalent bond. In addition to drug delivery applications, polycationic polymers have applications as DNA condensing agents for gene delivery[16,17] and as attachment factors that promote cell adhesion to conventional culture substrates[18] (e.g., glass or plastic).

Ranger et al. reported the synthesis of N-protected poly(hydroxyproline ester) (PHP ester),[10] consisting of naturally occurring hydroxyproline. Hydroxyproline is a constituent of collagen, gelatin, and other proteins.[19,20] Polymer made from hydroxyproline is expected to be useful in biomedical applica-

* Address correspondence to this author at Center for Molecular Catalysis (CMC), Department of Chemistry, Seoul National University, San 56-1, Shilim-dong, Kwanak-ku, Seoul 151-742, Korea. Phone: 82-2-880-6660. Fax: 82-2-889-1568. E-mail: pfjspark@plaza.snu.ac.kr.

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tions with minimum toxicity because the degradation product is a natural metabolite.

Here we report the synthesis of PHP ester from *N*-benzyloxycarbonyl (cbz)-protected poly(hydroxyproline ester) in which the reversible cbz protection group can be removed by catalytic transfer hydrogenation. After deprotection, there remain side-chain amine groups, which make the PHP ester water-soluble and become positive under the near neutral pH. We used matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) as a method to investigate degradation of polycationic, water-soluble, and biodegradable PHP ester. As PHP ester has polycationic nature, we investigated the interaction of the polymer with polyanionic polymer, DNA, to assess the possibility of using it in gene delivery, a biodegradable biomedical application, and other possible uses.

Experimental Section

Materials and Reagents. *N*-cbz-4-hydroxy-L-proline, formic acid, *N*-[2-hydroxyethyl]piperazine-*N*′-[2-ethanesulfonic acid] (Hepes), and DNase I were purchased from Sigma Chemical Co. Sinapinic acid, α-cyano-4-hydroxy cinnamic acid (αCHCA), potassium chloride, D_2O, and chloroform-d_1 were from Aldrich Chemical Co. Wizard Maxipreps DNA purification system for the purification of plasmid DNA was from Promega.

Measurements. ^{1}H NMR spectra were recorded on a Bruker DPX-300 NMR spectrometer. Infrared spectra were recorded with a Bruker IFS 48 FT-IR spectrophotometer. Samples were pressed into KBr pellets. Gel permeation chromatography (GPC) was carried out using a Waters model 600E Multi Solvent Delivery System, a model 410 refractive index detector, and Autochro-GPC software (Young-In Scientific, Korea) for data acquisition with Styragel HR 5E and three columns placed in series. Chloroform was used as the eluent at a flow rate of 0.5 mL/min. The molecular weights were determined relative to narrow molecular weight polystyrene standard from Waters. The MALDI experiments were done on a Voyager Biospectrometry workstaion (Perseptive Biosystems, Inc.) in the linear mode. A N_2 laser radiating at 337-nm wavelength with 3-ns pulses was used. The ions generated by the laser pulses were accelerated to 28-kV energy.

Synthesis of Polymer. Polymerization was conducted as described[10] with slight modifications. A Pyrex tube equipped with a sidearm was charged with 5 g of monomer (*N*-cbz-4-hydroxy-L-proline) and then fitted with a dry ice/acetone trap. After melting of the monomer, a magnetic drive unit with an impeller and a propeller was inserted into the tube. With gentle stirring, high vacuum (10^{-4} mmHg) was applied at 120 °C. Temperature was increased to 180 °C over 3 h. After 5 days of reaction, the crude polymer was dissolved in chloroform and purified by precipitating it into a large excess of methanol. After purification, 3.8 g of fine powder of poly(4-hydroxy-*N*-cbz-L-proline ester) (PHCP ester) was obtained. Deprotection of the cbz group was done as described.[15] Briefly, PHCP ester was dissolved in DMF, and Pd−C was added as a catalyst. With vigorous stirring, formic acid was slowly added. The reaction was continued for 15 h. After the replacement of formate salt by HCl, the concentrated solution was precipitated into a large excess of acetone, resulting in a white powder of PHP ester.

PHP ester: IR ν 2946 (N−H), 1751 (C═O, ester), 1245 (C−C(═O)−O); ^{1}H NMR (300 MHz, D_2O) δ 5.69 (1H, br s, CH), 4.83 (1H, br m, CH), 3.81 (2H, br m, CH_2), 2.63 (2H, br m, CH_2).

Regeneration of cbz Protecting Group. To a slurry of PHP ester (50 mg) and triethylamine (68 mg, 0.67 mmol) in methylene chloride (5 mL) at 0 °C was added benzyl chloroformate (85 mg, 0.5 mmol). After the mixture was stirred 30 min at 0 °C, it was further reacted for 30 min at room temperature. The reaction mixture became clear after the reaction. The reaction mixture was concentrated under a stream of nitrogen gas and precipitated into a large excess of methanol. The precipitate was dried and analyzed by GPC.

Molecular Weight Measurement by MALDI-TOF MS. A matrix solution of sinapinic acid was prepared at a concentration of 10 mg/mL in water/3% trifluoroacetic acid (TFA)/acetonitrile, 4:1:5 (v/v). KCl was dissolved in methanol at a concentration of 10 mg/mL. For MALDI analysis, PHP ester in water at a concentration of 5 mg/mL was mixed with sinapinic acid and KCl solution at a volumetric ratio of 1:1:2. A 1-μL aliquot was applied to the MALDI sample plate and dried in a vacuum.

Degradation Study. PHP ester was dissolved in 25 mM Hepes buffer to make pH 7 at a concentration of 5 mg/mL. The temperature of the PHP ester solution was adjusted to 37 °C. At an appropriate time interval, 1 μL of the solution was sampled and mixed with sinapinic acid and KCl solution. αCHCA solution was prepared at a concentration of 10 mg/mL in water/3% TFA/acetonitrile, 4:1:6 (v/v). One microliter of PHP ester solution was mixed with 9 μL of αCHCA solution. The solution pH change was measured at an appropriate time interval by a pH meter (Orion, MA). All the spectra were obtained at the same laser power.

Electrophoretic Mobility Shift Assay. PHP ester/DNA complexes were formed by mixing 200 μL of pSV-CAT plasmid solution (50 μg/mL in H_2O) with a 200-μL solution of 10-fold increasing concentration of PHP ester in Hepes-buffered saline (15 mM Hepes, 150 mM NaCl, pH 7.3) (HBS). After 1 h for the complex formation, samples were electrophoresed through a 0.8% agarose gel at 70 V for 40 min and stained with ethidium bromide to visualize DNA.

Dynamic Light Scattering and ζ Potential. The *Z*-average particle size and polydispersity index of the PHP ester/DNA complexes were determined by dynamic light scattering (DLS) at 25 °C with a Malvern 4700 system using a 25-mW He−Ne laser (λ = 633 nm) as the incident beam at a scattering angle of 90° and Automeasure version 3.2 software (Malvern Instrument Ltd., UK). For data analysis, the viscosity (0.8905 mPa·s) and refractive index (1.333) of pure water at 25 °C were used. The sample was prepared by mixing each solution of 50 μg/mL DNA (pSV-CAT) in H_2O and 7.5 mM PHP ester solution in HBS.

The electrophoretic mobility of the particles was determined in a PC-4 cell with a Malvern ζ-sizer 2C unit (Malvern Instrument Ltd., UK) at 25 °C. The instrument was calibrated using a latex with known ζ potential. For ζ potential measurement, PHP ester concentration was increased gradually with fixed DNA concentration (25 μg/mL).

Nuclease Resistance of PHP Ester/DNA Complexes. PHP ester/DNA complexes were formed by mixing a solution of plasmid, pSV-CAT (50 μg/mL), 200 μL in H_2O, with 7.5 mM PHP ester, 200 μL in HBS. After 1 h of incubation at room temperature, 10 units (1 μL) of DNase I (Sigma) were added to the tube and mixed well. The absorbance change at 260 nm was measured at a 30-s interval.

Cell Culture and Transfection. CPAE cells, a calf pulmonary arterial endothelial cell line, were grown in minimum essential medium with 2 mM L-glutamine, Earle's BSS adjusted to contain 1.5 g/L sodium bicarbonate, 0.1 mM nonessential amino acids, and 1.0 mM sodium pyruvate, and 20% fetal bovine serum (FBS). The cells were seeded at a density of 10^4 cells/well in 24-well plate, 24 h prior to transfection. Due to its rapid degradation in aqueous condition, PHP ester was dissolved in water just prior to mixing with plasmid DNA. PHP ester/pSV-β-gal complexes were prepared by mixing PHP ester and pSV-β-gal in FBS-free cell culture medium and incubated for 20 min at room temperature for the complex formation. Chloroquine was added at a final concentration of 100 μM. Medium in the 24-well plate was replaced with transfection mixture followed by 4 h of incubation at 37 °C. The transfection mixture was then replaced with fresh medium containing 20% FBS. Cells were further incubated for 48 h at 37 °C. The β-galactosidase activity in transfected cells was determined using colorimetric ONPG (*o*-nitrophenyl β-D-galactopyranoside) assay system (Promega, Madison, WI).

Results and Discussion

Polymer Synthesis. To synthesize biodegradable, biocompatible, and polyamine-containing polycationic polymer, 4-hydroxy-L-proline was chosen as a monomer since it is a major constituent of collagen, gelatin, and other proteins.[19,20] Synthesis of the polymer was done by a melting condensation polymerization (Scheme 1). The obtained polydispersity (1.7) of PHCP ester was reasonable for a melting condensation polymer (Table 1). Complete removal of the cbz group from PHCP ester was

Scheme 1

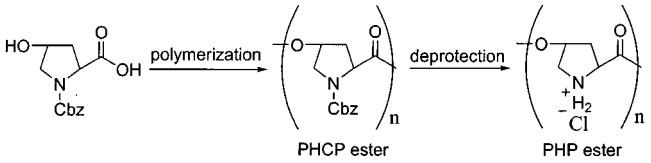

Table 1. Characterization of PHCP Ester and cbz Protecting Group-Regenerated Polymer

method	result
IR (KBr, cm^{-1})	2951, 2887 (C−H), 1750 (C═O, ester), 1708 (C═O, amide), 1419 (C−N), 1176 (C−C(═O)−O), 755, 697 (C−H, phenyl)
^{1}H NMR (300 MHz, $CDCl_3$)	7.35 (5H, m, Ph), 5.36 (1H, br s, CH), 5.13 (2H, br m, CH_2), 4.41 (1H, br m, CH), 3.76 (2H, br m, CH_2), 2.36 (2H, br m, CH_2)
^{13}C NMR (75 MHz, $CDCl_3$)	35.09 and 51.99 (2 **C**H_2's), 57.65 (**C**HC(═O)−O), 67.23 (**C**H_2Ph), 73.30 (**C**H−O−C(═O)), 127.67−128.26 (3° aromatic C's), 135.90 (4° aromatic C), 154.41 (carbamate carbonyl), 170.81 (ester carbonyl)
M_n (DP[b])	5300 (21)
M_w (DP)	9000 (36)
PD[c]	1.7
M_n (DP) after cbz regeneration	6600 (27)
M_w (DP) after cbz regeneration	8400 (34)
PD after cbz regeneration	1.3

[a] Molecular weight was determined relative to polystyrene standard by GPC. [b] Degree of polymerization. [c] Polydispersity.

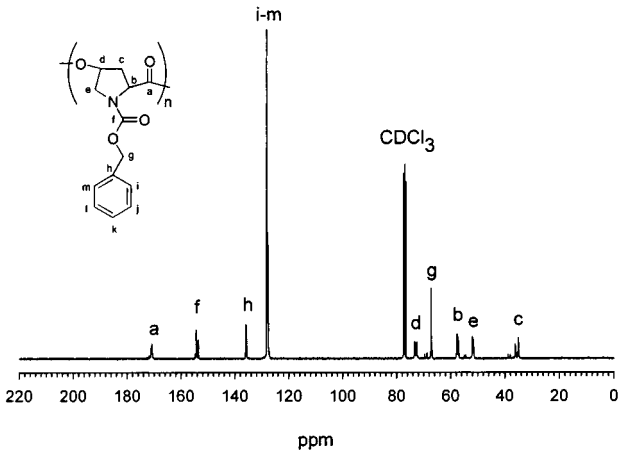

Figure 1. ^{13}C NMR spectrum of PHCP ester in $CDCl_3$.

confirmed by the disappearance of the phenyl peak at 7.35 ppm in the ^{1}H NMR spectrum upon deprotection (data not shown). Spectroscopic results from FT-IR (Table 1), ^{1}H NMR (Table 1), and ^{13}C NMR (Table 1 and Figure 1) were in agreement with the proposed structure of PHCP ester. Small peaks in the ^{13}C NMR spectrum at 37.11, 52.80, and 67.84 ppm seem to be from end groups. To investigate the change of degree of polymerization (DP) upon deprotection, secondary amine protecting cbz group was regenerated from PHP ester. GPC analysis shows that the number- and weight-average DPs of cbz-regenerated polymer were 27 and 34, respectively (Table 1). Those of PHCP ester were 21 and 36, respectively. This minute change of the DP shows that the integrity of the polymer backbone is largely preserved under deprotection conditions. The number- and weight-average DPs after regeneration became larger and smaller, respectively. And polydispersity (PD) was narrower after regeneration. As esterification is a reversible

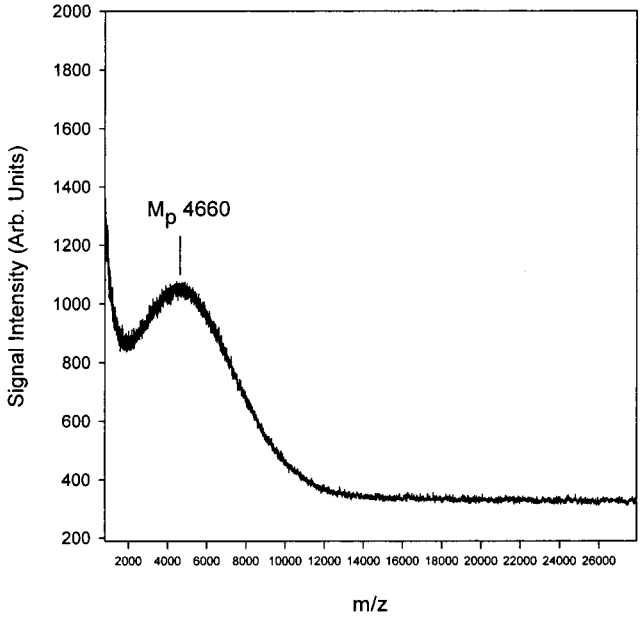

Figure 2. MALDI-TOF mass spectrum of PHP ester. The spectrum was obtained in the continuous extraction mode. Sinapinic acid was used as matrix and KCl as cationizing reagent. The spectrum is the sum of 32 laser shots, and it was 7-point Savizky−Golay smoothed.

reaction, the cleavage and formation of an ester bond can occur simultaneously under acidic (due to formic acid) deprotection conditions. We think that the overall result of a reversible esterification reaction makes the PD narrower and the number- and weight-average DPs larger and smaller, respectively.

Molecular Weight Measurement and Degradation Study in Aqueous Solution. The molecular weight of PHCP ester was determined by GPC because it is soluble in organic solvent, but the cbz group-deprotected polymer, PHP ester, is soluble in water. The problems associated with traditional methods for molecular weight distribution (MWD) measurement, e.g., gel permeation chromatography for accurate measurement of molecular weight and molecular weight distribution of water-soluble polymers, is well known.[21] We tried to determine the molecular weight of PHP ester by GPC with poly(ethylene glycol) as a standard, but the measured molecular weight was only about 600. We were not able to obtain the appropriate standard, e.g., a narrow polycationic polymer, commercially, so we tried to measure the molecular weight of PHP ester by MALDI-TOF MS. MALDI is a mild ionization process. With the use of an appropriate matrix and sample preparation protocol, high-molecular-weight polymers with masses up to 1.5 million Da can be analyzed.[22] MALDI-MS is a powerful tool for analyzing the molecular weights of some polymers, e.g., poly(ethylene glycol) and poly(styrene).[23] But determination of the molecular weights of strongly basic or strongly acidic components is difficult or impossible by MALDI.[24] PHP ester is a strongly basic polymer. By using sinapinic acid as a matrix and KCl as a cationizing reagent, we were able to obtain the MALDI-MS spectrum (Figure 2). From the fact that no spectrum was obtained without KCl, it seems that KCl has a crucial role as a cationizing reagent in ionizing PHP ester. The ratio of PHP ester, sinapinic acid, and KCl was important in obtaining the spectrum.

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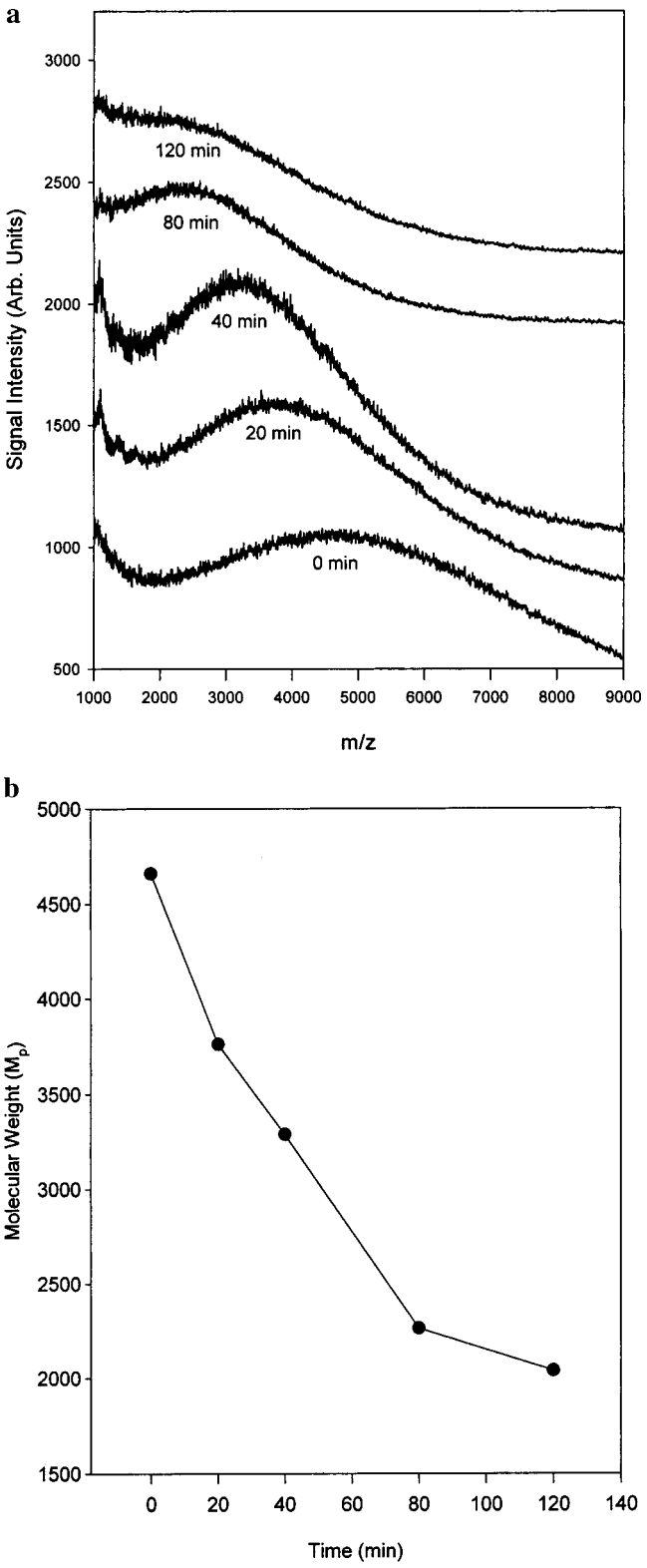

Figure 3. (a) MALDI-TOF spectra of PHP ester degradation. MALDI spectra of PHP ester at a 37 °C buffer condition (pH 7) were obtained by varying the incubation time. The times (in min) represent incubation time. The spectrum is the sum of 32 laser shots, and it was 7-point Savitzky−Golay smoothed. (b) Plot of M_p change as a measure of PHP ester degradation.

The degradation study of PHP ester was performed at pH 7.0, 37 °C. It is well known that the MWD of synthetic polymers having a PD greater than 1.1 cannot be accurately characterized by MALDI-MS alone because of mass discrimination effects.[25,26] So we determined not to describe molecular weight by M_n or M_w, which are commonly used to describe polymer average molecular weights. But the tendency toward degradation as a result of MWD change could easily be monitored by using M_p, the most probable peak molecular weight determined from the highest peak intensity in the MALDI-MS spectrum. In an aqueous solution of pH 7, it is shown that the polymer degraded to less than half the molecular weight of intact polymer in less than 2 h (Figure 3). As the pH of the solution was decreased or increased, PHP ester was degraded slower or faster, respectively (data not shown). Such a rapid degradation of PHP ester seems to have mainly from two causes: first, hydrolysis by water, and second, secondary amine groups in each monomeric unit acting as nucleophiles. A degradation study of biodegradable polyester such as poly(lactide-*co*-glycolide) (PLGA) shows that it needs 2−24 months to be degraded.[27] PLGA is composed of an ester in the backbone with no pendant functional groups. Hydrolysis by water is the main reason for the degradation of PLGA.[28] As PHP ester degraded much faster compared to PLGA, it seems that PHP ester is degraded mainly by the attack of its own amine group. As more amine groups are free of protonation as pH increases, PHP ester will be degraded more quickly. The degradation of PHP ester may also be due to hydrolysis by water, but hydrolysis by its own amine groups seems to be the main reason for the degradation within 1 day. There have been reports about the syntheses of "self-destroying polymers" by the introduction of pendant carboxylic acid groups to polyesters.[29,30] As an amine group has a stronger nucleophilicity than a carboxylic acid group, it is expected that the degradation of polyester with pendant amine groups would be faster than that of polyester with pendant carboxylic acid groups.

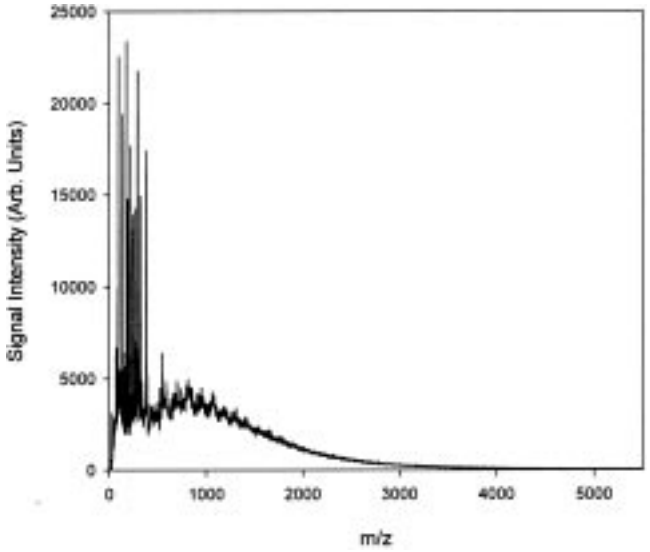

Figure 4. PHP ester degradation after 2 days of incubation. The MALDI spectrum was obtained by using αCHCA as matrix. The spectrum is the sum of 32 laser shots, and it was 7-point Savitzky−Golay smoothed.

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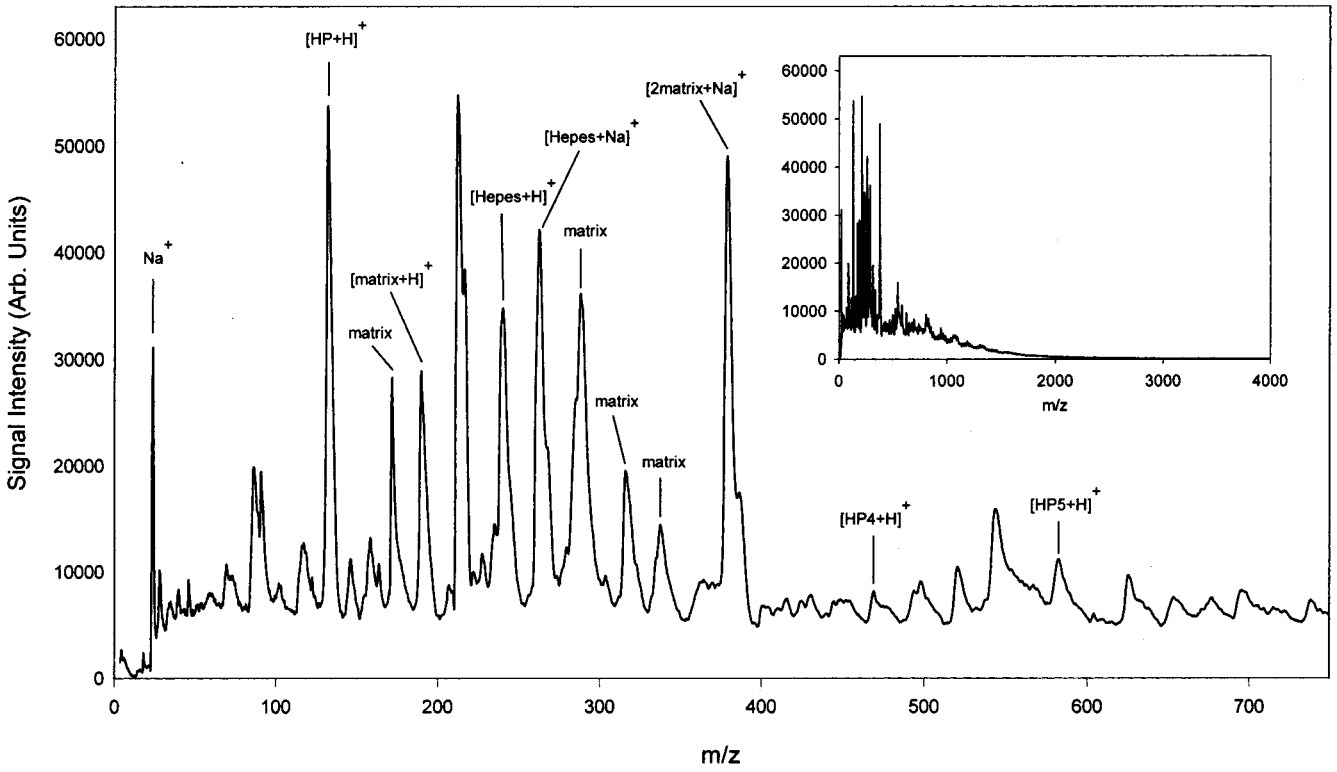

Figure 5. Degradation of PHP ester to near completion. The PHP ester solution as described above was incubated at 37 °C for 3 months. The MALDI spectrum was measured by using αCHCA as a matrix. HP, hydroxyproline; HP2, dimer of hydroxyproline; HP4, tetramer of hydroxyproline; HP5, pentamer of hydroxyproline; matrix, peak found in αCHCA background. The full spectrum is shown in the upper right position. The spectrum is the sum of 128 laser shots, and it was 7-point Savitzky−Golay smoothed.

Figure 4 shows the MALDI spectrum of PHP ester after 2 days of incubation. The spectrum was obtained by using αCHCA as a matrix because αCHCA is generally a more efficient matrix in ionizing low-molecular-weight compounds than sinapinic acid.[31] There were no significant differences between the two matrixes in MALDI spectra other than signal intensities (data not shown). MWD was similar to the 1-day incubation spectrum (data not shown). This result represents the fact that PHP ester was not degraded as fast as it was during the initial stage (within 1 day). The MWD changed very slowly until 3 months. After 3 months, PHP ester was degraded nearly completely to its monomeric unit, hydroxyproline (Figure 5). PHP ester was degraded very fast at the initial stage, but after this period PHP ester was degraded very slowly until it was degraded completely to the monomeric unit.

Because PHP ester consists of an ester bond, degraded products will have carboxylic acids, which will lower the pH of the solution. Degradation of PHP ester in a pH 7 buffered condition at 37 °C was monitored by measuring the pH change of the solution. The pH of the solution changed drastically to a lower value within 1 day (Figure 6). But after this period, the pH change became slower until 3 months. This result is in agreement with the MALDI degradation data, in that PHP ester degraded very fast at the initial stage, but degradation became slower after this period (Figures 3 and 4).

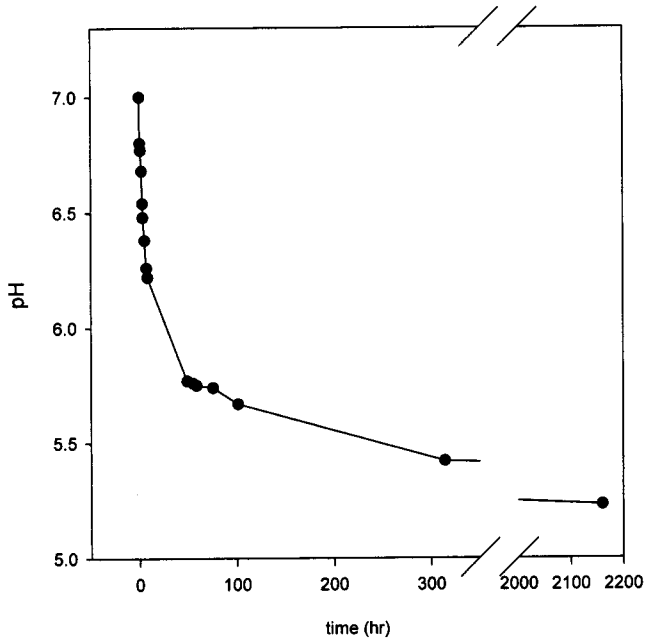

Figure 6. pH change profile during incubation of PHP ester. The pH change of PHP ester solution as described in the Experimental Section was monitored with time.

Condensation of Polyanion by Polycationic PHP Ester To Make Self-Assembled Complexes. To study the interaction of PHP ester with DNA, we performed electrophoretic mobility shift assay (EMSA) as shown in Figure 7a. DNA alone is shown in lane 1, showing four DNA bands corresponding to the four forms of the plasmid DNA: super-coiled, nicked circular, and dimers of each of these forms. As the proportion of PHP ester in the samples increased (evident in lanes 2−6), there was an increase in DNA that remained at the top of the gel with the PHP ester. DNA was wholly retained in the well at lane 6. This result represents the fact that amine groups of PHP ester with positive charges interact with DNA phosphate groups with negative charges to form neutral self-assembled complexes. We incubated PHP ester/DNA complexes for 4 h at 37 °C. As shown

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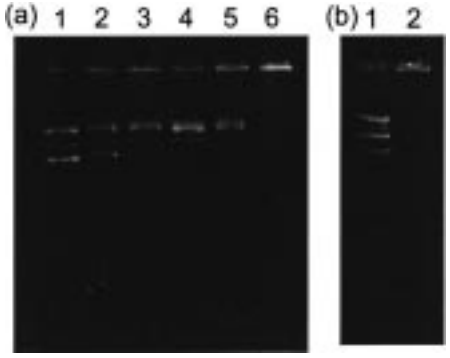

Figure 7. Effect of increasing proportions of PHP ester on plasmid DNA electrophoretic migration (EMSA). (a) Lane 1, DNA alone; lane 2, 7.5×10^{-4} mM PHP ester; lane 3, 7.5×10^{-3} mM PHP ester; lane 4, 7.5×10^{-2} mM PHP ester; lane 5, 7.5×10^{-1} mM PHP ester; lane 6, 7.5 mM PHP ester in HBS. (b) Lane 1, DNA alone; lane 2, the complexes were incubated for 4 h at 37 °C after the complexes' formation (7.5 mM PHP ester).

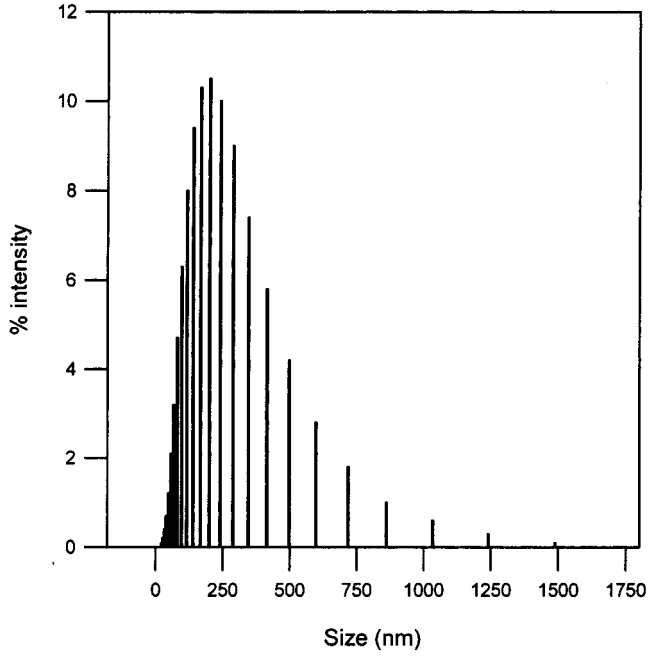

Figure 8. Dynamic light scattering of PHP ester/DNA complexes. Size distribution of PHP ester/DNA complexes was measured by DLS. The concentrations of PHP ester and DNA for the complex formation were used as determined by EMSA (Figure 7).

in Figure 7b, the PHP ester/DNA complexes band is clearly seen retaining whole DNA inside the well. This explains the fact that the complexes are stable for at least 4 h at 37 °C. It seems that partially degraded PHP ester retains the ability to make a complex with DNA, or PHP ester is more slowly degraded when it is complexed with DNA than when it is not. If the latter is true, it will result from the fact that amine groups of PHP ester are fully occupied by the phosphate groups of DNA.

The size distribution of PHP ester/DNA complexes was measured by DLS (Figure 8). The complexes had an average diameter of 194 nm as determined by DLS. The size distribution was rather dispersed, as demonstrated by the polydispersity index (0.48).

Figure 9 shows the results of the ζ potential of PHP ester/DNA complexes. As expected, naked DNA possesses a negative ζ potential (-21 mV). In the presence of PHP ester, a positive ζ potential was observed. The concentration of PHP ester needed

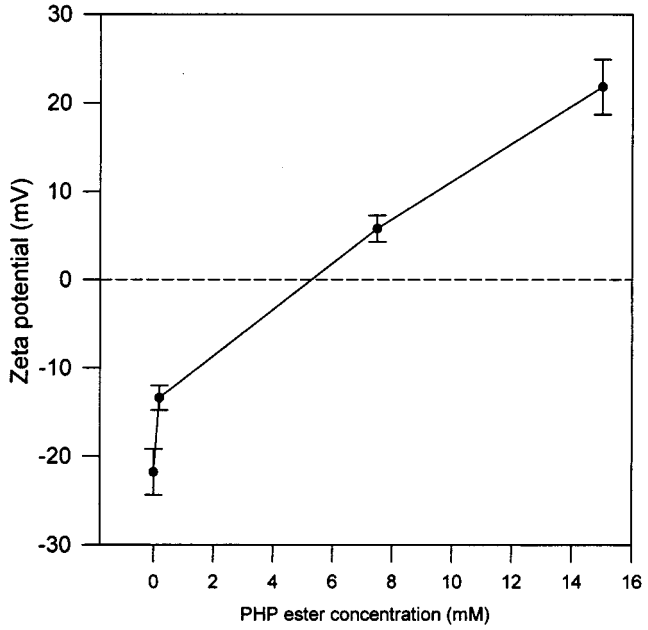

Figure 9. ζ potential of PHP ester/DNA complexes at a fixed DNA concentration (25 μg/mL) and an increasing PHP ester concentration. The complexes were allowed to form for 1 h at room temperature before measurements. The results are expressed as mean values ($\pm$SD) of three measurements.

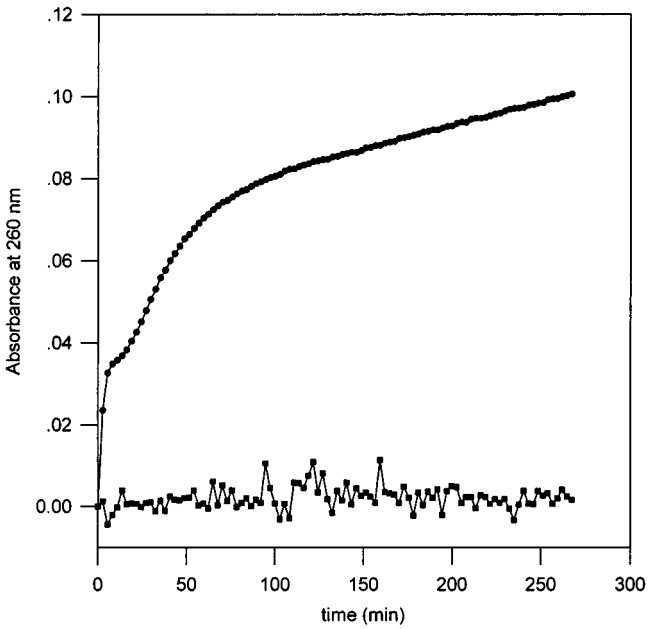

Figure 10. Nuclease resistance of PHP ester/DNA complexes. The change of absorbance for DNA or PHP ester/DNA complexes was monitored at 260 nm after DNase I (10 units) addition. (●) DNA alone; (■) PHP ester/DNA complexes.

to form PHP ester/DNA complexes is consistent with the EMSA data (Figure 7). Obviously, the plasmid is becoming fully occupied with PHP ester molecules at high PHP ester concentration to form neutral PHP ester/DNA complexes.

When DNA is attacked by nuclease, absorbance at 260 nm is increased, due to fragmentation of the DNA.[32] We observed the protection of DNA against nuclease (DNase I) attack when the PHP ester/DNA complexes were formed (Figure 10). Although native DNA was fragmented rapidly in less than 2 min by nuclease, PHP ester/DNA complexes were stable through

(32) Katayose, S.; Kataoka, K. *Bioconjugate Chem.* **1997**, *8*, 702−707.

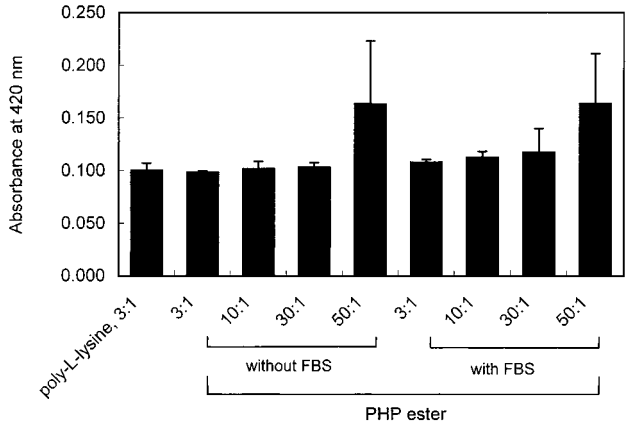

Figure 11. Transfection efficiency of PHP ester/pSV-β-gal complexes into CPAE cells. Lane 1, 20% FBS was added; lane 2−5, FBS was not added in transfection mixtures; lane 6−9, 20% FBS was added. Numbers indicate weight ratio (polymer:DNA). The cells were incubated with polymer/DNA complexes containing varying amounts of polymer and a fixed amount (5 μg) of the plasmid DNA. Chloroquine was used at a concentration of 100 μM in all transfection mixtures. Data are expressed as mean values (±SD) of four independent experiments.

4 h. The high nuclease resistance ability of PHP ester/DNA complexes indicates that nuclease is not able to find enough free substrate (DNA), as DNA is bound strongly to PHP ester. This also indicates that PHP ester is not degraded enough to lose its ability to make PHP ester/DNA complexes, and it is not degraded as quickly as when it is not bound to DNA.

Delivery and Expression of Foreign Gene into Mammalian Cells by PHP Ester/DNA Complexes. To assess the possibility of PHP ester as a gene delivery carrier, PHP ester/pSV-β-gal complexes were transfected into CPAE cells (Figure 11). The transfection efficiency of PHP ester was comparable to that of poly-L-lysine,[16,17] which is the most common polymer for gene delivery. As the amount of PHP ester was increased relative to that of DNA, the transfection efficiency was increased. The presence of FBS did not alter the transfection efficiency of PHP ester. These results showed that PHP ester could be used as a gene delivery carrier.

Conclusions

We synthesized a novel self-destroying, biodegradable, and polycationic polymer, poly(4-hydroxy-L-proline ester), with hydroxyproline, a major constituent of collagen, gelatin, and other proteins, as a repeating unit. PHP ester is likely to be nontoxic because this polymer is degraded very fast and completely to hydroxyproline. There have been reports about the synthesis of biodegradable polycationic polymers,[7,12,15] but very few of them have dealt with their applications. The degradation study we performed for water-soluble, biodegradable polymer by MALDI-TOF MS seems to be the first such study to our knowledge. MALDI-TOF MS will be useful in investigating degradation of water-soluble, fast-degrading polymers. By using MALDI-TOF MS, molecular weight of degraded monomer as a degradation product as well as polymer molecular weight can easily be monitored. We investigated the interaction of PHP ester with DNA, as PHP ester is a polycation, and assessed the possibility of using it in polyanion (such as DNA) condensation. The PHP ester formed soluble polymer/DNA complexes with average diameters of less than 200 nm. Although PHP ester was degraded very quickly when it was alone in solution, it was more stable when complexed with DNA. Finally, PHP ester complexed with DNA could transfect mammalian cells, showing the possibility of using the polymer as a gene delivery carrier. PHP ester has potential as a biomaterial in delivering negatively charged compounds (e.g., drugs or DNA) or in other applications that need biodegradable polycationic polymer (e.g., attachment factors that promote cell adhesion to conventional culture substrates), etc.

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