Water-Soluble Biodegradable Polyphosphazenes Containing *N*-Ethylpyrrolidone Groups

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Received May 3, 2005; Revised Manuscript Received July 8, 2005

ABSTRACT: Novel water-soluble biodegradable polyphosphazenes are described. Polymers are constructed on the basis of a biodegradable polyphosphazene backbone to mimic the structure of poly(vinylpyrrolidone), PVP. Poly{di[2-(2-oxo-1-pyrrolidinyl)ethoxy]phosphazene}, PYRP, and its copolymers containing biologically active carboxylatophenoxy side groups have been synthesized. The ability of these polymers to degrade in aqueous environment at 37 °C is demonstrated by light scattering-GPC and NMR studies. The degradation is manifested by the gradual decrease in the molecular weight and release of the side group −1-(2-hydroxyethyl)-2-pyrrolidone. It has been shown that the incorporation of *N*-ethylpyrrolidone containing side groups in the polymer structure can modulate degradation profiles of phosphazene copolymers. The kinetics of hydrolytic degradation and the effect of pH on the degradation rate have been investigated.

Introduction

Water-soluble synthetic macromolecules present significant interest as polymers for biomedical applications. Poly(vinylpyrrolidone) (PVP), in particular, has been extensively studied in pharmaceutical formulations, drug delivery systems, biocompatible coatings, and as a plasma expander.[1−4] High molecular weight PVP is also of interest; however, its applications are severely limited, since it can accumulate in the body and cause undesirable side effects.[4] This problem can be addressed potentially through the development of biodegradable polymers containing *N*-ethylpyrrolidone groups.

Interest in polymers with a phosphazene backbone stems from the remarkable properties of their main chain. Some polyphosphazenes undergo hydrolytical degradation in an aqueous environment, which makes them attractive candidates for biological applications. However, the selection of such macromolecules is limited and most of the hydrolytically unstable polyphosphazenes synthesized to date are not soluble either in water or in polyelectrolytes.[5,6]

The goal of this study is to develop water-soluble polymers that can combine the biologically relevant properties of neutral *N*-ethylpyrrolidone groups and the advantages of a biodegradable polyphosphazene backbone. The present paper describes synthesis of biodegradable polyphosphazenes with *N*-ethylpyrrolidone groups and studies on their hydrolytical degradation in aqueous solutions.

Experimental Section

Materials. Hexachlorocyclotriphosphazene, trimer (Nippon Fine Chemicals, Japan) was used as received. The macromolecular precursor, PDCP, was synthesized using ring-opening polymerization of hexachlorocyclotriphosphazene in a titanium pressure reactor as described previously.[7] 1-(2-Hydroxyethyl)-2-pyrrolidone, 98% (Aldrich) was used as received; propyl 4-hydroxybenzoate (propyl paraben), 99.5+% (Aldrich) and sodium propyl paraben, USP/NF grade (Spectrum Quality Products, Gardena, CA) were dried prior to use in a vacuum oven at 80 °C for 2 h. Sodium hydride, dry, 95%, and 2-methoxyethyl ether (diglyme), anhydrous, 99.5% (Aldrich) were used as received. Poly[di(carboxylatophenoxy)phosphazene], PCPP, was synthesized as described previously.[8]

Synthesis of Poly{di[2-(2-oxo-1-pyrrolidinyl)ethoxy]phosphazene}, PYRP. A suspension of sodium hydride (0.066 g; 0.0026 mol) in 1,4-dioxane (0.0008 L) was slowly added to 1-(2-hydroxyethyl)-2-pyrrolidone (1.59 g; 0.0123 mol) in 1,4-dioxane (0.010 L) under nitrogen to form sodium 1-(2-hydroxyethyl)-2-pyrrolidone. This solution was diluted with 0.015 L of 1,4-dioxane and then heated to 50 °C with stirring under anhydrous nitrogen. 0.002 L of polydichlorophosphazene solution in diglyme (0.116 g; 0.001 mol) was then added slowly via syringe over a period of five minutes. The reaction mixture was stirred for fifteen hours at 50 °C, then cooled to ambient temperature and precipitated with hexane. The precipitate was collected by decantation, dried under vacuum, and then dissolved in water. The polymer then was purified using a Biocad Perfusion chromatography workstation (Applied Biosystems, Foster Hills, CA) equipped with a Modcol CER 3662 column using 0.02 M ammonium bicarbonate. Polymer fractions were collected and then lyophilized. The yield was 0.113 g (37.5% of theoretical). Polymer was characterized by GPC, ^{1}H NMR and ^{31}P NMR.

Synthesis of Poly{[2-(2-oxo-1-pyrrolidinyl)ethoxy](4-carboxylatophenoxy)phosphazene}, Copolymers CP1 and CP2. Two approaches were used to synthesize mixed substituent copolymers: (a) nucleophilic reagents were mixed and then reacted with poly(dichlorophosphazene) to form copolymer CP1, and (b) the nucleophilic reagents were added to poly(dichlorophosphazene) in a sequential order to prepare copolymer CP2.

In a method (a), a suspension of sodium hydride (0.086 g; 0.003 mol) in 1,4-dioxane (0.002 L) was slowly added to a solution of 1-(2-hydroxyethyl)-2-pyrrolidone (2.08 g; 0.016 mol) in 0.010 L of 1,4-dioxane under nitrogen to form sodium 1-(2-hydroxyethyl)-2-pyrrolidone. Sodium propyl 4-hydroxybenzoate was prepared by adding a suspension of sodium hydride (0.61 g; 0.025 mol) in diglyme (0.004 L) to a solution of propyl ester of 4-hydroxybenzoic acid (5.19 g; 0.029 mol) in 0.010 L of diglyme under dry nitrogen. 0.0005 L of the sodium propyl 4-hydroxybenzoate solution was added to the sodium 1-(2-hydroxyethyl)-2-pyrrolidone solution at room temperature. 0.002 L of polydichlorophosphazene solution in diglyme (0.116 g; 0.001 mol) was then added slowly at ambient temperature over a period of five minutes. Subsequently, the temperature

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10.1021/ma0509309 CCC: $30.25
Published on Web 08/17/2005

was raised to 50 °C and the reaction mixture was stirred for fifteen hours. 0.001 L of 12.7 N aqueous potassium hydroxide solution was slowly added, and the reaction mixture was kept at 50 °C for 1 h with stirring. The precipitate was collected by decantation, dried, and dissolved in water. The polymer then was purified chromatographically as described above. The yield of copolymer CP1 was 0.228 g (75% of theoretical).

In a method (b), a suspension of sodium hydride (0.247 g; 0.0098 mol) in 1,4-dioxane (0.105 L) was slowly added to 1-(2-hydroxyethyl)-2-pyrrolidone (5.989 g; 0.0464 mol) in 1,4-dioxane (0.025 L) under a dry nitrogen to form sodium 1-(2-hydroxyethyl)-2-pyrrolidone. Sodium propyl 4-hydroxybenzoate was prepared by adding a suspension of sodium hydride (0.707 g; 0.0279 mol) in diglyme (0.003 L) to propyl ester of 4-hydroxybenzoic acid (6.055 g; 0.3360 mol) in diglyme (0.010 L) under a dry nitrogen atmosphere. 0.003 L of polydichlorophosphazene solution in diglyme (0.232 g; 0.002 mol) was diluted with 0.013 L diglyme at room temperature under nitrogen and then heated to 50 °C. To this solution 0.0024 L of the sodium propyl 4-hydroxybenzoate suspension was added while stirring. The temperature was increased to 100 °C; the reaction mixture was stirred for 3 h and then cooled to 50 °C. To this 0.015 L of the sodium 1-(2-hydroxyethyl)-2-pyrrolidone solution was slowly added and the reaction continued for twenty hours. 0.010 L of 12.7 N potassium hydroxide solution was slowly added, and stirred for 1 h at 50 °C. The precipitated polymer was collected, dissolved in distilled water, and precipitated by adding 1 N hydrochloric acid to pH 3. The precipitate was redissolved in 0.05 M ammonium bicarbonate and purified as described above. The yield of copolymer CP2 was 0.29 g (38.6% of theoretical).

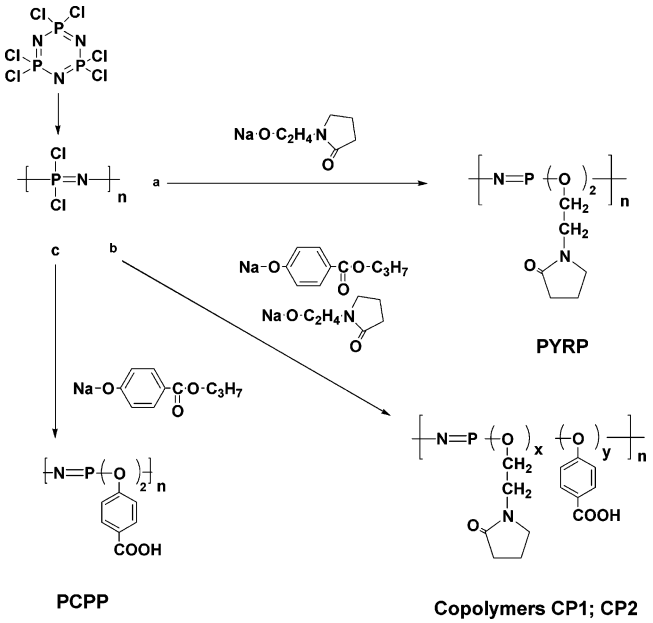
Analytical Methods. ^{31}P, ^{13}C, and ^{1}H NMR spectra were recorded using Bruker 400 NMR spectrometer. D_2O was used as a solvent.

The HPLC system was configured as follows: Shodex DEGAS KT-37 on-line vacuum degasser (Showa Denko K. K., Tokyo, Japan); Waters 600 HPLC pump (Waters, Milford, MA); two inline filters − 0.5 micron high-pressure filter (Rainin, Woburn, MA) and 0.02 micron filter (Anodisc 25, Whatman International Limited, Maidstone, England) in a high-pressure stainless filter holder (Millipore, Bedford, MA); Waters 717plus autosampler; ultrahydrogel linear column (Waters, Milford, MA), a MALLS detector (DAWN DSP−F, Wyatt Technology, Santa Barbara, CA); and a Waters 996 photodiode array detector, and a Waters 410 refractive index detector. PBS (pH 7.4) was used as a mobile phase, the flow rate was 0.75 mL/min. Mobile phase was filtered through a 0.02 μm filter (Anodisc 47, Whatman International, Maidstone, England) into a four-valve ULTRA-WARE filtration reservoir (Kontes, Vineland, New Jersey). 0.01% sodium azide was added to mobile phase to prevent biological degradation of the columns. The molecular weight of the polymer was determined as described previously.[9]

Analysis of Copolymer Composition. The composition of mixed substituent copolymers was determined using two methods. (1) It was calculated based on the ratio between the peak areas of ethylene protons of the *N*-ethylpyrrolidone group and the aromatic protons of the carboxylatophenoxy side group in ^{1}H NMR. (2) The composition was established using HPLC based on the differences in the UV absorbance of PCPP and PYRP at 254 nm in PBS (pH 7.4). Calibration curves were obtained for the mixtures of PCPP and PYRP by plotting HPLC peak areas at 254 nm versus mixture composition. The total polymer concentration was maintained at 1 mg/mL and the results were processed using Millenium (Waters, Milford, MA) software. Copolymer was then analyzed by HPLC using the same conditions, and its molar composition was determined using calibration curves obtained for the mixtures of homopolymers.

Degradation Studies. The solutions of polymers PYRP, CP1, CP2, and PCPP were prepared at 1 mg/mL concentrations in citrate buffer, pH 3.0 (0.040 M citric acid; 0.021 M sodium hydroxide; 0.060 M sodium chloride), Tris buffer, pH 7.4 (0.020 M Tris, 0.9% NaCl), and borate buffer, pH 9.3 (0.02 M sodium tetraborate). To achieve sink conditions, samples

Scheme 1. Synthesis of *N*-Ethylpyrrolidone Containing Polyphosphazenes (a, b) and PCPP (c)



were incubated in buffer solution at room temperature for 1 h at a concentration of 5 mg/mL upon shaking to ensure complete dissolution, diluted 5-fold, and then filtered using 0.45 μm Millex-HV syringe filters (Millipore, Bedford, MA).

Degradation studies were performed at 55 °C. Vials containing polymer solutions were incubated in a G24 environmental incubator shaker (New Brunswick Scientific, Edison, NJ). 0.2 mL samples were collected periodically for the determination of molecular weight and degradation products. Analysis was conducted using size exclusion HPLC with multi-angle laser light scattering, UV photodiode array, and refractive index detection systems. Absolute molecular weight parameters were determined using light scattering detection, refractive index detector as a mass detector, and ASTRA 2.1 software (Wyatt Technology, Santa Barbara, CA). Relative molecular weight characteristics were calculated based on calibration curves for poly(ethylene oxide) standards using photodiode array detection with absorbance measured at 230 nm. Concentration of hydroxybenzoic acid (HBA) was determined by HPLC with UV detection at 254 nm using Millenium (Waters, Milford, MA) software. Phosphate buffered saline PBS, pH 7.4, was used as a mobile phase with a flow rate of 0.75 mL/min and an injection volume of 0.1 mL.

Results and Discussion

Synthesis of PYRP. Polymer synthesis was conducted by reacting poly(dichlorophosphazene) with 1-(2-hydroxyethyl)-2-pyrrolidone activated with sodium hydride (Scheme 1). Initial experiments using dioxane, tetrahydrofuran, and diglyme as solvents failed to generate high molecular weight polymer. This was attributed to the low solubility of sodium 1-(2-hydroxyethyl)-2-pyrrolidone in these solvents, which are typically used for the macromolecular substitution reaction. To improve its solubility in the reaction mixture 1-(2-hydroxyethyl)-2-pyrrolidone was added as a cosolvent to 1,4-dioxane (5-fold excess of 1-(2-hydroxyethyl)-2-pyrrolidone compared to the sodium activated form). The reaction was conducted at 50 °C.

Synthesis of Mixed Substituent Copolymers. Mixed substituent copolymers containing ionic moieties were also synthesized (Scheme 1). Polyphosphazene polyelectrolytes, such as PCPP, have been previously

Table 1. NMR Characterization Data

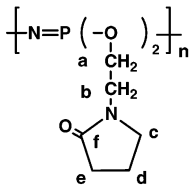
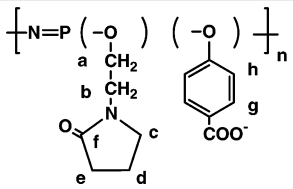
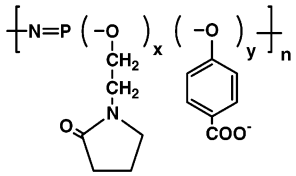
Polymer	NMR, ppm	
 **PYRP**	^{1}H	2.02 (**d**); 2.38 (**e**); 3.49 (**b**); 3.51 (**c**); 4.03 (**a**)
	^{13}C	178.11 (**f**); 63.65 (**a**); 48.61 (**b**); 43.11 (**c**); 30.79 (**e**); 17.78 (**d**)
	^{31}P	-7.4
 **CP1; CP2**	^{1}H	1.97 (**d**); 2.37 (**e**); 3.41 (**b**); 3.47 (**c**); 3.95 (**a**); 7.25 (**h**); 7.94 (**g**)
	^{31}P	-19.7; -13.7; -7.4

Table 2. Polymer Compositions and Molecular Weights

Polymer					
	Composition, x : y			M_w, g/mol x 10^{-3}	
	Expected *	^{1}H NMR	UV-HPLC	MALLS-GPC #	GPC (PEO) Δ
PYRP	2 : 0	2 : 0	2 : 0	370	305
CP 1	1.70 : 0.30	1.70 : 0.30	1.64 : 0.36	1000	610
CP 2	0.3 : 1.70	0.18 : 1.82	0.18 : 1.82	513	471

[a] Based on the composition of the reaction mixture. [b] Based on GPC with multi angle laser light scattering detection. [c] Based on GPC with PEO standards.

demonstrated to be potent immunostimulants.[10−13] Thus, copolymers of PYRP and PCPP were prepared to study the effect of *N*-ethylpyrrolidone containing side groups on the degradation profile of these biologically important polyphosphazenes. Two synthetic approaches were used: (a) nucleophilic reagents were mixed and then reacted with poly(dichlorophosphazene) to form copolymer CP1, and (b) nucleophilic reagents were added to poly(dichlorophosphazene) in a sequential order to prepare copolymer CP2.

Polymer Characterization. Since polyphosphazenes are prepared via the macromolecular substitution route, a critical parameter in the synthesis of a new polymer is a completion of the substitution reaction. ^{31}P NMR spectra of PYRP showed a single peak, indicating that no detectable chlorine atoms are present in the polymer and no side reactions leading to structural irregularities in the polymer took place. Thus, substitution conditions were adequate for the completion of the reaction and did not lead to the pyrrolidone ring-opening reactions. The structure of PYRP was confirmed by ^{1}H and ^{13}C NMR, which also demonstrated purity of the polymer and the absence of detectable byproducts in the polymer structure. Chemical shifts for PYRP and CP1 are presented in Table 1.

All synthesized polymers had molecular weights in excess of 300 000 g/mol, indicating the absence of significant hydrolytical degradation during the synthesis (Table 2). Copolymer composition was determined by both ^{1}H NMR and HPLC using differences in the UV absorption of the side groups. Table 2 shows good correlation between two different methods in the determination of copolymer composition.

PYRP was found to be soluble in water, methanol, ethanol, DMF, DMSO, acetonitrile, and dioxane, and not soluble in diglyme, ethyl acetate, and hexane.

Light Scattering HPLC Studies of Polymer Hydrolysis. Hydrolytic degradation of the synthesized polymers was conducted in aqueous solutions. No polymer cross-linking or precipitation was observed in any of the studied systems. Figure 1 shows the kinetics of weight average molecular weight decrease of PYRP in aqueous solutions with pH 9.3, pH 7.4, and pH 3.0 at 55 °C. It appears that the rate of hydrolysis is pH dependent, with the degradation accelerating as pH declines. This relationship is observed for both absolute molecular weight, determined by GPC with multi-angle laser light scattering detector (Figure 1a), and relative molecular weight, measured by GPC using PEO standards (Figure 1b). The higher hydrolytical sensitivity

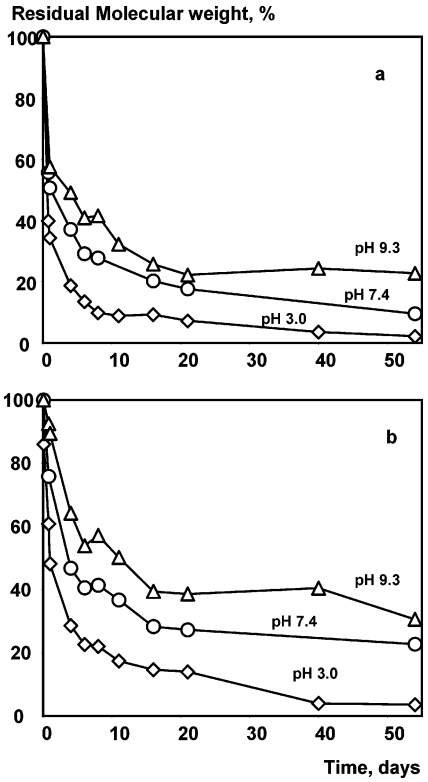


Figure 1. Molecular weight loss of PYRP in aqueous solutions at various pH versus time. (55 °C, polymer concentration: 1 mg/mL; weight average molecular weight determined by: (a) light scattering; (b) GPC with PEO standards).

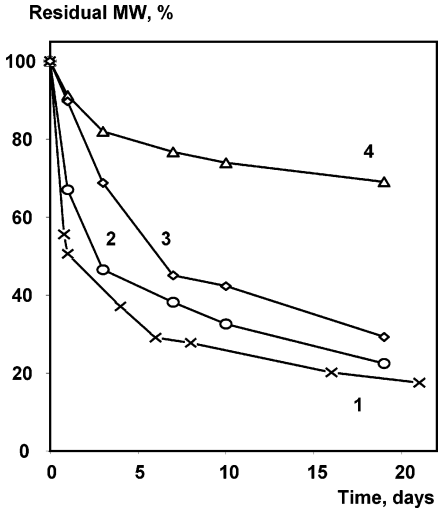


Figure 2. Molecular weight loss of PYRP (1), CP1 (2), CP2 (3), and PCPP (4) in aqueous solution versus time (weight average molecular weight determined by light scattering; PBS, pH 7.4; 55 °C, polymer concentration − 1 mg/mL).

of polymer in the acidic environment is interesting from the biological standpoint and can open opportunities for the design of systems that can potentially discriminate between extracellular fluids and endosomes.[6]

Degradation profiles for PYRP, CP1, CP2, and PCPP were also studied (Figure 2). Pyrrolidone containing homopolymer demonstrated the highest hydrolysis rate, and PCPP the lowest. Most importantly, addition of pyrrolidone side groups to the PCPP structure resulted in a pronounced increase in the rate of molecular weight loss (CP1). The degradation of PCPP and its copolymer CP2 was also accompanied with a side group cleavage, manifested in the release of hydroxybenzoic acid, HBA

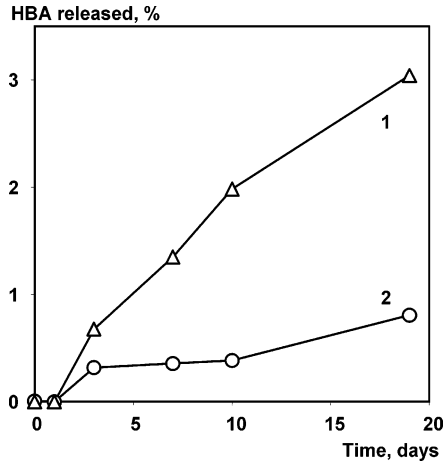


Figure 3. Release of *p*-hydroxybenzoic acid in aqueous solutions for CP1 (1) and PCPP (2) versus time (55 °C, 1 mg/mL).

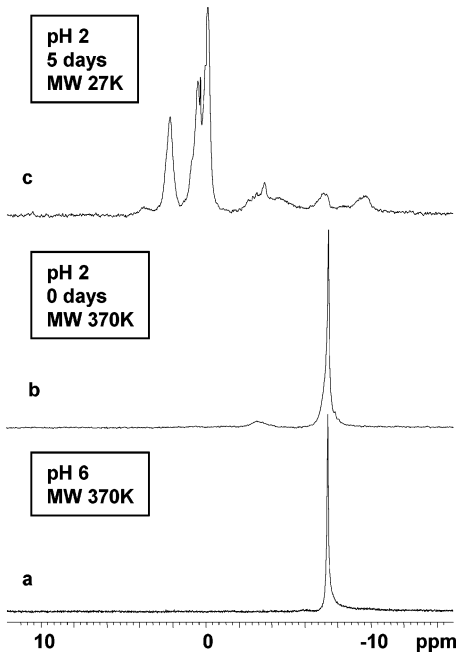
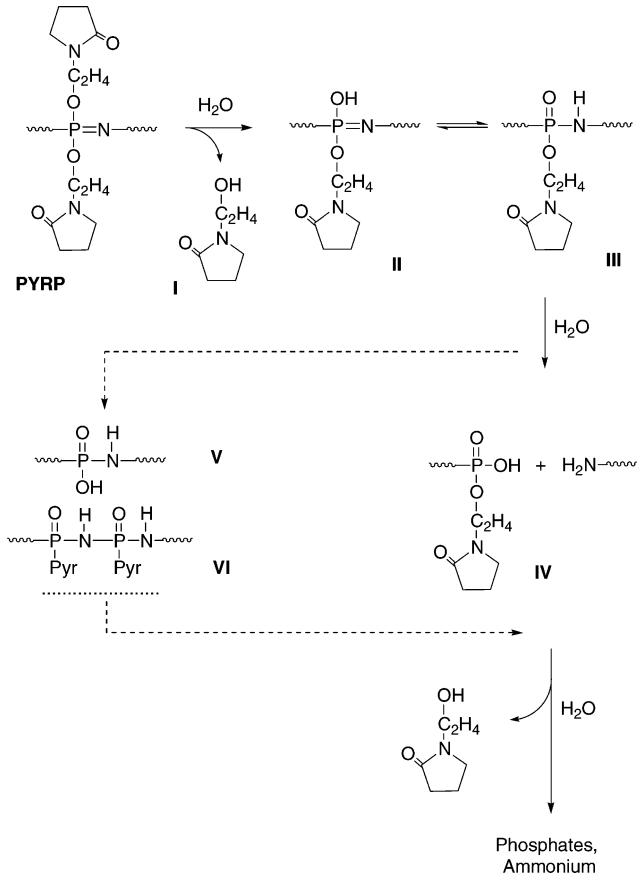


Figure 4. ^{31}P NMR spectra of PYRP in D_2O, pH 6 (a), in D_2O after addition of deuterated hydrochloric acid, pH 2 (b), and after incubation of PYRP in D_2O − deuterated hydrochloric acid, pH 2 at 55 °C for 5 days (c). Molecular weights, determined by light scattering, were 370 000 g/mol (a, b) and 27 000 g/mol (c).

(Figure 3). Interestingly, the rate of HBA release was higher for CP1 than for PCPP, which contains more carboxylatophenoxy groups. This also confirms a destabilizing effect of pyrrolidone side groups. The results demonstrate the potential of *N*-ethylpyrrolidone containing side groups in modulating polyphosphazene degradation.

NMR Studies of the Hydrolysis Process. Figure 4 shows changes in the ^{31}P NMR spectra observed during the hydrolysis of PYRP in the acidic aqueous environment. As mentioned earlier, the ^{31}P spectrum of PYRP in water (pH 6) contained one broad singlet at −7.4 ppm (Figure 4a). Admixing of hydrochloric acid to this system immediately resulted in the appearance of additional peaks, indicating that polymer hydrolysis proceeds rapidly under acidic conditions (Figure 4b). In an attempt to assign the spectra, the order of signal appearance was monitored and the results were cor-

Scheme 2. Possible Hydrolytic Pathways for Polyphosphazenes with *N*-Alkyl Pyrrolidone Groups



related with the data on the hydrolysis study of poly(dichlorophosphazene) and hexachlorocyclotriphosphazene.[14,15] Based on this strategy, the peak at −3.2 ppm can be ascribed to P−OH; signals and −9.8, −7.8, and −8.0 ppm can be assigned to PyrO−P−OPyr in α, β, and γ positions to P−OH. The fact that these resonances grew in intensity with time supports this assumption. The ratio between the intensities of α-PyrO−P−OPyr (−9.8 ppm) and PyrO−P−OPyr (−7.4 ppm) peaks also increased dramatically in the course of the hydrolysis. Peaks in the range of −1 to 4 ppm (Figure 4c) probably can be associated with low molecular weight phosphate, oligomers, and products of geminal hydrolysis. Observed changes in the ^{31}NMR spectra were accompanied by the decrease in the weight average molecular weight of the polymer from 370 000 g/mol (Figure 4b) to 27 000 g/mol (Figure 4c).

^{1}H NMR analysis of the polymer hydrolyzed in the acidic solution for 5 days still showed original macromolecular peaks of PYRP, which was expected since light scattering analysis demonstrated the presence of polymer with the molecular weight of 27 000 g/mol. In addition, 1-(2-hydroxyethyl)-2-pyrrolidone was identified, indicating the release of the side groups. Approximately 70% of the theoretical amount of 1-(2-hydroxyethyl)-2-pyrrolidone was released from the polymer after 5 days of the hydrolysis. No other compounds were detected, demonstrating the absence of ring opening reactions.

Possible Hydrolysis Pathways. The mechanism of polyphosphazene hydrolysis has been discussed previously.[5,6] Initial steps in the suggested pathways include (a) a reaction between side groups and the backbone (intramolecular catalysis) or (b) hydrolytic cleavage of the side group. Unlike previously described polyphosphazenes containing tertiary amino groups or amino acids, in which side groups can potentially catalyze the hydrolysis process, polyphosphazene with ethylpyrrolidone side groups was not expected to have an intramolecular mechanism of degradation. Our inability to detect products of the ring-opening reactions using ^{1}H NMR speaks in favor of this assertion. Scheme 2 shows a potential hydrolytic pathway based on the mechanism (b). Hydrolytical cleavage of the side group leads to the experimentally observed release of 1-(2-hydroxyethyl)-2-pyrrolidone (**I**), formation of hydroxyl derivative (**II**), and unstable phosphazane structure (**III**), culminating in the breakdown of the polymer chain (**IV**). The formation of products resulting from the geminal (**V**) and nongeminal hydrolysis (**VI**) is also possible. As suggested previously, the final polyphosphazene degradation products can also include ammonium and phosphate ions.[5]

Conclusion

Novel biodegradable water-soluble polyphosphazenes containing *N*-ethylpyrrolidone groups have been synthesized. Hydrolytic degradation of these polymers in aqueous solutions is manifested by the gradual decrease in the molecular weight and cleavage of the side groups. The rate of hydrolysis is pH sensitive, with more rapid polymer breakdown occurring in the acidic environment. The possible degradation pathways are discussed. *N*-ethylpyrrolidone containing side groups can be used to modulate the degradation rate of mixed substituent polyphosphazenes, including biologically active polyelectrolytes.

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MA0509309