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Synthesis of Poly(organophosphazenes) with Glycolic Acid Ester and Lactic Acid Ester Side Groups: Prototypes for New Bioerodible Polymers

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ABSTRACT: Four different poly(organophosphazenes) bearing esters of glycolic or lactic acid as side groups have been synthesized in order to investigate their stability in aqueous media. The polymers are poly[bis(ethyl glycolato)phosphazene], poly[bis(ethyl lactato)phosphazene], poly[bis(benzyl glycolato)phosphazene], and poly[bis(benzyl lactato)phosphazene]. The benzyl esters of glycolic and lactic acid were prepared by the base-catalyzed transesterification of their ethyl esters with benzyl alcohol at 90 °C. Poly(dichlorophosphazene) was treated with a large excess of the appropriate sodium acid ester at 50 °C to yield the fully-substituted polymer. To avoid unwanted molecular weight decline, these polymers were stored under nitrogen or in vacuum. Hydrolytic decomposition profiles in aqueous media were monitored by ^{31}P NMR spectroscopy and by gel permeation chromatography (GPC). The possible utility of these polymers as biomedical materials is discussed.

Introduction

Hydrolytically-sensitive polymers have been used in numerous medical applications. Two of these polymers, poly(glycolic acid) and poly(lactic acid), are widely used as surgical sutures and in other biomedical devices.[1,2] Both of these polymers are crystalline materials that hydrolyze at a controlled rate when exposed to aqueous media. Through earlier work in our laboratory, we have shown that polyphosphazenes that bear amino acid ester (1 and 4), imidazolyl (3), glyceryl (2), or glycosyl (5) side groups are also hydrolytically-sensitive.[3-9] The structures of these polymers are shown in Chart 1. Recently, the synthesis and degradation behavior of polyphosphazenes bearing the ethyl lactate esters of glycine or alanine as side groups has been reported.[10,11]

In the present study, a series of new polyphosphazenes with pendent esters of glycolic or lactic acid as side units have been prepared. These polymers are poly[bis(ethyl glycolato)phosphazene] (**6**), poly[bis(ethyl lactato)phosphazene] (**7**), poly[bis(benzyl glycolato)phosphazene] (**8**), and poly[bis(benzyl lactato)phosphazene] (**9**). In this study, the hydrolytic stability of these polymers was investigated in solution and in the solid state. In both investigations, the molecular weight decreased significantly in the presence of water. The decomposition products of the hydrolysis reactions were identified as ethanol or benzyl alcohol, glycolic or lactic acid, phosphates, and ammonia. These products can be easily metabolized and, when present in small quantities, are nontoxic.

Synthesis of Polymers 6–9. Poly(dichlorophosphazene) was allowed to react with a large excess of the sodium salt of ethyl glycolate (**10**), ethyl lactate (**11**), benzyl glycolate (**12**), or benzyl lactate (**13**) to produce the fully-substituted polymers **6–9**. The syntheses of these polymers are shown in Scheme 1.

Three problems were encountered in the synthesis of these polymers. First, the sodium salts of the glycolate and lactate esters are only marginally soluble in organic media and can be used only as dilute solutions in tetrahydrofuran (THF). Sodium ethyl lactate remained soluble in THF only at concentrations of 0.43 M or lower. This insolubility generated problems for carrying out the chlorine replacement reactions. The second difficulty involved the selection of a suitable reaction temperature.

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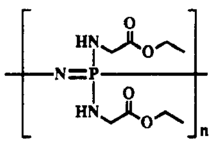
Chart 1



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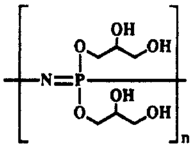

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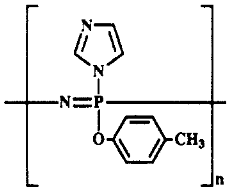

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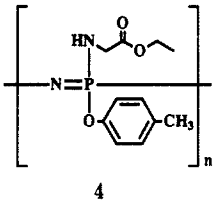

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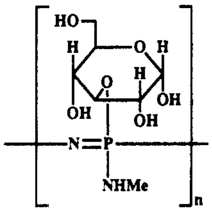

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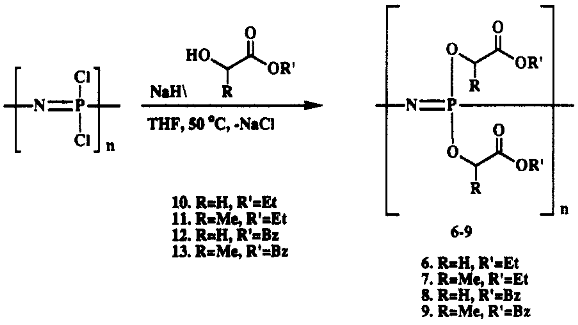
Scheme 1



In refluxing THF (66 °C), poly(dichlorophosphazene) reacted initially with each of the reagents, but, after 72 h of exposure to the nucleophiles, phosphorus–nitrogen backbone cleavage occurred before chlorine replacement was complete. On the other hand, if these reactions were carried out at room temperature in THF, skeletal cleavage reactions were insignificant but polymers **6–9** remained only partially substituted after 2 weeks. A temperature of 50 °C optimized the substitution reaction in THF and minimized polymer backbone cleavage. The final challenge was the removal of the sodium chloride produced during the reaction of poly(dichlorophosphazene) with the sodium salts of the acid esters. After experiments using dialysis, precipitation into organic solvents, and Soxhlet extraction, it was determined that the removal of the salts was most efficiently accomplished by multiple precipitations from THF into isopropyl alcohol. During and after the purification procedure, polymers **6–9** were stored under dry nitrogen or in vacuum to avoid exposure to water.

These macromolecules were then subjected to molecular structure characterization. The characterization data are shown in Table 1. These data are consistent with the structures shown for **6–9**. In particular, no evidence was found from the NMR spectra that side group "diesters" had been formed through an attack by the sodium alkoxide on the ester function. Polymer **6** is an elastomeric material, while polymers **7–9** are brittle glasses. No crystalline melting transitions were detected for polymers **6–9** by differential scanning calorimetry experiments or by cross-polarized optical microscopy.

Polymer Hydrolysis Studies. Polymers **6–9** underwent hydrolysis when exposed to water. The decomposition reactions were monitored in solution and in the solid state. In the solution hydrolysis studies (with the polymers dissolved in THF with 5% deionized water), the reactions were monitored by ^{31}P NMR spectroscopy and by gel permeation chromatography (GPC). The time required for the onset of polymer hydrolysis was estimated by ^{31}P NMR spectroscopy from the time required for a resonance to appear in the ^{31}P NMR spectrum at approximately 0.0 ppm (phosphates). This occurred after 270 h for polymer **6** and 466 h for polymer **7**. Poly[bis(benzyl glycolato)phosphazene] (**8**) showed evidence of initial decomposition after 587 h in contact with the THF/water mixture, while poly[bis(benzyl lactato)phosphazene] (**9**) showed detectable degradation after 980 h in the THF/water solution.

Polymer solution hydrolysis was also monitored by gel permeation chromatography, after 1, 3, 5, and 7 weeks of exposure to water. In solution, polymers **6–9** showed a broadening of the GPC peak followed by a significant decline in molecular weight. These data are shown in Figure 1. However, a consistent variation in hydrolysis behavior based on changes in side group structure was not observed. Poly[bis(ethyl glycolato)phosphazene] (**6**) showed the greatest decline in molecular weight over the monitored time period. Compared to each other, polymers **7** and **8** showed similar losses in molecular weight. However, polymer **8** hydrolyzed at a slightly higher rate than polymer **7**. Poly[bis(benzyl lactato)phosphazene] (**9**) decomposed at the slowest rate of the four polymers.

In the solid-state hydrolysis experiments, the reactions were also monitored by ^{31}P NMR spectroscopy and by

Table 1. Characterization Data for Polymers 6–9

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polymer	^{31}P NMR (ppm)	^{1}H NMR (ppm)	M_w	T_g (°C)		% C	% H	% N	% Cl
6	-7.8	4.4 (2H)	1.1×10^5	0	calcd	38.24	5.58	5.58	0
		4.2 (2H)			found	37.68	5.48	5.62	0.26
		1.3 (3H)							
7	-10.6	4.4 (1H)	2.7×10^5	58	calcd	43.01	6.45	5.02	0
		4.2 (2H)			found	43.22	6.51	4.93	0.52
		1.6 (3H)							
		1.4 (3H)							
8	-8.7	7.4 (5H)	1.0×10^5	43	calcd	57.60	4.80	3.73	0
		5.2 (2H)			found	58.04	4.71	3.85	0.41
		4.6 (2H)							
9	-9.6	7.4 (5H)	1.2×10^5	84	calcd	59.55	5.46	3.47	0
		5.3 (2H)			found	59.16	5.52	3.56	0.56
		4.4 (1H)							
		1.4 (3H)							

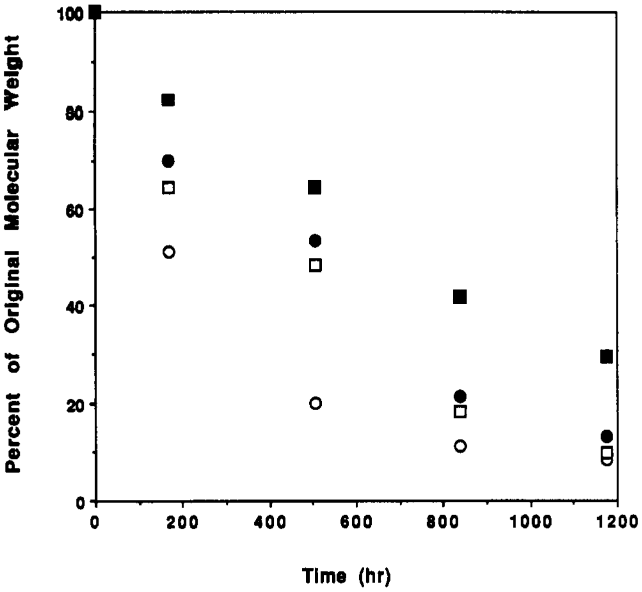

Figure 1. Molecular weight ($M_{GPC} \simeq M_w$) changes during the solution hydrolysis of polymers (6–9) in aqueous media at 37 °C: (○) poly[bis(ethyl glycolato)phosphazene] (6); (●) poly[bis(ethyl lactato)phosphazene] (7); (□) poly[bis(benzyl glycolato)phosphazene] (8); (■) poly[bis(benzyl lactato)phosphazene] (9).

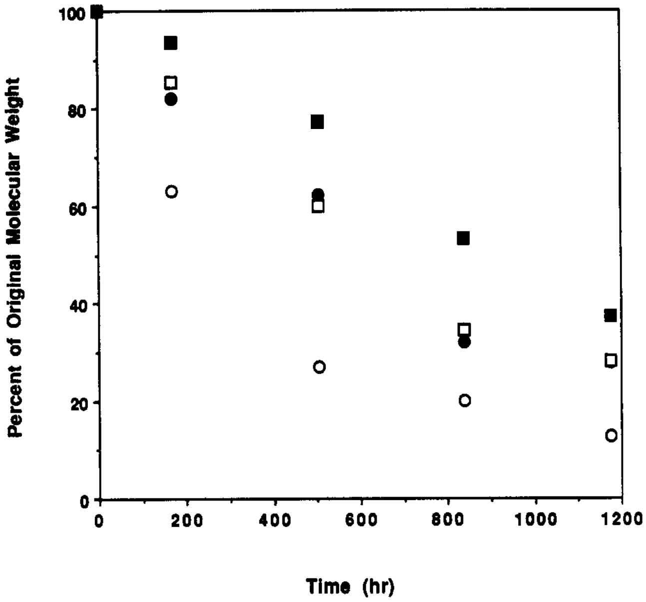

Figure 2. Molecular weight (M_{GPC}) changes during the solid-phase hydrolysis of polymers (6–9) in aqueous media at 37 °C: (○) poly[bis(ethyl glycolato)phosphazene] (6); (●) poly[bis(ethyl lactato)phosphazene] (7); (□) poly[bis(benzyl glycolato)phosphazene] (8); (■) poly[bis(benzyl glycolato)phosphazene] (9).

GPC. In these studies, ^{31}P NMR spectra of the aqueous media surrounding polymers 6–9 were examined. As with the solution studies, the spectra in these experiments also showed the appearance of a sharp singlet at approximately 0.0 ppm after a specific time for each polymer. This occurred at 320 h for polymer 6 and 525 h for polymer 7. Polymer 8 showed evidence of initial backbone hydrolysis after 656 h in contact with the aqueous media. Poly[bis(benzyl lactato)phosphazene] (9) showed initial evidence of decomposition after 1107 h.

In the solid-state decomposition studies, polymers 6–9 showed a decline in molecular weight as determined by GPC over time. These data are shown in Figure 2. The graph illustrates that a distinct pattern exists for the solid-phase hydrolysis of the polyphosphazenes. Poly[bis(ethyl glycolato)phosphazene], polymer 6, decomposed at the fastest rate. Again, this result is consistent with the fact that 6 is the least sterically encumbered species of the four polymers. Thus, water molecules can probably displace ethyl glycolato side units or hydrolyze the ester function to the acid. The pendent carboxylic acid could induce skeletal degradation. Polymers 7 and 8 had nearly identical solid-phase decomposition rate profiles. Polymer 7, poly[bis(ethyl lactato)phosphazene], possesses a methyl group on the α-carbon of its side groups, whereas poly[bis(benzyl glycolato)phosphazene], polymer 8, bears bulky, hydrophobic benzyl ester groups. Each of these features could hinder hydrolytic attack at the phosphorus atoms in the polymer chain. Thus, the molecular weight decline of these two polymers was not as rapid as detected for polymer 6. Polymer 9, poly[bis(benzyl lactato)phosphazene], showed the slowest solid-phase molecular weight decline over time. Again, this slow decomposition rate can be explained in terms of steric effects. Polymer 9 has both of the hydrolysis-limiting factors of polymers 7 and 8, specifically the methyl group on the α-carbon as well as the benzyl ester.

For those polyphosphazenes with benzyl ester side units, hydrolytic cleavage of the polymer backbone required a longer time in both the solution and the solid-state hydrolysis investigations. This result can be explained by the fact that the ethyl esters are probably more hydrophilic than the benzyl esters. Moreover, the polymers bearing the lactato side groups degraded at a slower rate than the polyphosphazenes bearing glycolato side units with the same ester group. The lactato side units bear a methyl group on the α-carbon which would make such polymers less prone to hydrolytic cleavage than the glycolato-bound polyphosphazenes.

In addition to the hydrolytic sensitivity studies, the products of hydrolysis polymers 6–9 both from solution and solid-state studies were also identified. The hydrolysis products are benzyl alcohol or ethanol, glycolic or lactic acid, phosphates, and ammonia. Ethanol and benzyl alcohol were detected by ^1H NMR spectroscopy by monitoring the upfield shift of their methylene protons from ester to the alcohol. Glycolic and lactic acids were identified by mass spectrometry. Phosphates were detected by treatment of the hydrolysis media with zirconyl chloride or silver nitrate. All the media that contained the hydrolysis products gave either white zirconyl phosphate precipitates or yellow silver phosphate precipitates. Ammonia was detected by the ninhydrin test.

Polymers 6–9 as Potential Biomaterials. The objective of this work was to design and develop synthesis routes to materials that might behave like poly(glycolic acid) and poly(lactic acid) but have unique combinations of properties. However, unlike PGA and PLA, polymers 6–9 were not crystalline, as measured by differential scanning calorimetry or by cross-polarized optical microscopy. This may be a consequence of the high flexibility of the phosphazene backbone. Moreover, the ester groups of the side units may be sufficiently dynamic to disrupt packing interactions. Polymers 6–9 hydrolyzed at a faster rate than either PGA or PLA. The lack of crystallinity and the relatively rapid hydrolysis rates may be appropriate for controlled drug release applications. Moreover, mixed-substituent phosphazene polymers (i.e., poly[(ethyl lactato)(benzyl lactato)phosphazene]) can be prepared and would allow a "tailored" hydrolysis rate and release profile to be developed.

Experimental Section

Materials and Equipment. All synthesis reactions were carried out with the dry solvents and reactants protected by a stream of dry nitrogen (Matheson) using standard Schlenk line techiques. Tetrahydrofuran (Omnisolv) was distilled from sodium benzophenone ketyl under nitrogen. Hexane and diethyl ether (Fisher) were distilled from CaH_2 under nitrogen. Isopropyl alcohol, benzyl alcohol, ethyl glycolate, and ethyl lactate (Aldrich) were distilled from BaO and stored over 4-Å molecular sieves. Potassium hydroxide (85%; Fisher) and sodium hydride (60% dispersion in mineral oil; Aldrich) were used as received. Hexachlorocyclotriphosphazene (Ethyl Corp.) was recrystallized from hexane (3×) and then sublimed [30 °C (0.1 mmHg)]. Poly(dichlorophosphazene) was produced by the thermal ring-opening polymerization of hexachlorocyclotriphosphazene at 250 °C in a sealed, evacuated glass tube.[12] All ^{31}P NMR spectra were obtained using either a JEOL FX-90Q (36.2 MHz) or a Bruker WM-360 (145 MHz) NMR spectrometer. All ^{1}H NMR and ^{13}C NMR spectra were obtained using a Bruker WM-360 NMR spectrometer, operating at 360 and 90 MHz, respectively. Glass transition temperatures were determined by the use of a Perkin-Elmer DSC-7 with TAS-7 software. Molecular weights were estimated by gel permeation chromatography using a Hewlett Packard LC 1090 unit using a polystyrene stationary phase. Polystyrene standards were employed to calibrate the columns. Samples for GPC analyses were prepared in concentrations of approximately 1.5% (w/v) in THF. Elemental analyses were obtained from Galbraith Laboratories, Knoxville, TN.

Synthesis of Benzyl Glycolate (12) and Benzyl Lactate (13). The syntheses of compounds 12 and 13 were carried out in the same manner. The procedure for the preparation of benzyl lactate (13) is given as a typical example. Ethyl lactate (15.0 g, 0.12 mol) was dissolved in benzyl alcohol (65 mL, 0.62 mol). Potassium hydroxide (0.067 g, 85%) was then added to this mixture. After the potassium hydroxide had dissolved, the reaction mixture was heated to 90 °C. The ethanol was removed as the reaction proceeded and was collected in a Dean–Stark trap. After 4 h, a quantitative amount of ethanol (6.9 mL) had been collected. The excess benzyl alcohol was removed by distillation under vacuum [65 °C (0.1 mmHg)]. The crude product 13 was further distilled under vacuum [87 °C (0.1 mmHg)] to give pure product 13 as a clear, colorless liquid. Compound 12: ^{1}H NMR (360 MHz, $CDCl_3$) δ 7.4 (5H, s), 5.2 (2H, s), 4.2 (2H, s); ^{13}C NMR (99 MHz, $CDCl_3$) δ 175, 137, 129, 128, 127, 67, 66; MS by positive fast atom bombardment (+FAB). M(calcd): 166. M(found): 167. Compound 13: ^{1}H NMR (360 MHz, $CDCl_3$) δ 7.4 (5H, s), 5.2 (2H, s), 4.3 (1H, q), 2.8 (1H, s), 1.4 (3H, d); ^{13}C NMR (99 MHz, $CDCl_3$) δ 175, 136, 129, 128, 127, 68, 67, 22. MS by +FAB. M(calcd): 180. M(found): 181.

Synthesis of Polymers 6–9. The syntheses of polymers 6–9 were carried out using similar procedures. The synthesis of 7 is given as a typical example. Sodium hydride (17.2 g, 0.43 mol) was suspended in THF (800 mL). A solution of ethyl lactate (76.1 g, 0.645 mol) in THF (200 mL) was then added slowly to the suspension. When the reaction between ethyl lactate and sodium hydride was complete, poly(dichlorophosphazene) (10.0 g, 0.086 mol) was dissolved in THF (800 mL) and the salt solution was added to the polymer solution via an addition funnel. This mixture was then heated to approximately 50 °C for 96 h. The reaction mixture was cooled and concentrated under vacuum at room temperature. Polymer 7 was isolated and purified by two precipitations from THF into hexane, twice into diethyl ether, and five times into isopropyl alcohol. The polymer was then dried and stored under vacuum.

Solution Hydrolysis Studies. To dilute solutions of each polyphosphazene (0.5 g/100 mL of THF) was added 5.0 mL of distilled, deionized water. These solutions were exposed to the atmosphere and maintained at 37 °C with stirring. The ^{31}P NMR spectra were monitored every 72 h. The onset of hydrolytic decomposition of each polymer was determined by the appearance of a sharp resonance at 0.0 ppm in the ^{31}P NMR spectrum. The molecular weight ($M_{GPC} \simeq M_w$) decline of these samples was estimated by gel permeation chromatography after 1, 3, 5, and 7 weeks by direct analysis of the filtered reaction mixture.

Solid-State Hydrolysis of Polymers 6–9. Films (1 × 1 × 0.01 cm) cast from concentrated THF/acetone solutions of polymers 6–9 were used for these experiments. Two film samples of each polymer were immersed in distilled, deionized water at 37 °C without stirring. ^{31}P NMR spectra of the surrounding aqueous media were monitored every 72 h. At 1, 3, 5, and 7 weeks, the samples were removed, dried, and dissolved in THF. Gel permeation chromatography was then used to estimate their molecular weights and monitor the extent of backbone decomposition.

Detection of the Hydrolysis Products Formed in Aqueous Media. The hydrolysis products from both the solution and solid-state studies were examined. Phosphates were detected with the use of zirconyl chloride ($ZrOCl_2$). At several time intervals, $ZrOCl_2$ (1.5 mL of a 1.0 M solution) was added to aliquots removed from the aqueous media. Formation of a white precipitate of zirconyl phosphate indicated the presence of phosphate. Phosphates were also detected by the addition of $AgNO_3$ to aliquots of the hydrolysis medium to yield a yellow precipitate of silver phosphate. Glycolic and lactic acid were detected by mass spectrometry (+FAB). Ammonia was detected by the ninhydrin test.[13] A THF solution of ninhydrin (1.0 M, 5 mL) was added to the experiment media. An intense violet color appeared within minutes of the addition, which was taken to be evidence for the presence of ammonia. Benzyl alcohol and ethanol were identified by ^{1}H NMR spectroscopy (360 MHz). For benzyl alcohol, an aliquot of the hydrolysis medium was dried and the residue was dissolved in $CDCl_3$. The methylene protons of the benzyl alcohol (4.5 ppm) were detected. For identification of ethanol, an aliquot of the aqueous medium was heated to 90 °C to distill volatile species into a Dean–Stark trap. The liquid collected was dissolved in $CDCl_3$ and then analyzed by ^{1}H NMR spectroscopy (360 MHz). This analysis showed that ethanol was present (ethanol resonances at CH_2– 3.4 ppm (q), CH_3– 1.1 ppm (t)).

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