J|A|C|S
ARTICLES
Published on Web 04/17/2007

Tuning the Release Rate of Acidic Degradation Products through Macromolecular Design of Caprolactone-Based Copolymers

Minna Hakkarainen, Anders Höglund, Karin Odelius, and Ann-Christine Albertsson*

Contribution from the Department of Fibre and Polymer Technology, School of Chemical Science and Engineering, Royal Institute of Technology, S-100 44 Stockholm, Sweden

Received January 15, 2007; E-mail: aila@polymer.kth.se

Abstract: Macromolecular engineering is presented as a tool to control the degradation rate and release rate of acidic degradation products from biomedical polyester ethers. Three different caprolactone/1,5-dioxepan-2-one (CL/DXO) copolymers were synthesized: DXO/CL/DXO triblock, CL/DXO multiblock, and random cross-linked CL/DXO copolymer. The relation of CL and DXO units in all three copolymers was 60/40 mol %. The polymer discs were immersed in phosphate buffer solution at pH 7.4 and 37 °C for up to 364 days. After different time periods degradation products were extracted from the buffer solution and analyzed. In addition mass loss, water absorption, molecular weight changes, and changes in thermal properties were determined. The results show that the release rate of acidic degradation products, a possible cause of acidic microclimates and inflammatory responses, is controllable through macromolecular design, i.e., different distribution of the weak linkages in the copolymers.

Introduction

One of the key questions for the successful preparation of biocompatible, biodegradable, and resorbable synthetic polymers for biomedical applications is how to prepare materials with predetermined degradation rates and controlled release of degradation products. Among the most interesting materials are aliphatic polyesters derived from cyclic monomers, e.g., lactide (LA), glycolide (GA), and ϵ-caprolactone (ϵ-CL). Generally, good biocompatibility has been demonstrated for these polymers, but the release of acidic degradation products sometimes leads to acidic microenvironments and inflammatory responses. Presumably the effect of acidic degradation products on the surrounding tissue is negligible as long as the tissue clearance capacity is not disturbed. Large concentrations of degradation products may, however, cause complications and pH decrease and induce toxic responses. Especially if the degradation process proceeds with a burst mode (i.e., a sudden and rapid release of degradation products) or occurs in anatomical sites where there is minimal fluid flow, the body's ability to flush away the water-soluble degradation products may be overwhelmed.

Large concentrations of PLA degradation products had a toxic influence on cell culture systems.[1] Good biocompatibility was reported for up to 6 weeks after implantation of PLA into dog femurs.[2] However, after the degradation began, PLA induced inflammation and bone resorption. In a clinical study the most common postoperative problems after implantation of PLA/PGA rods were clinically manifested foreign-body reactions and osteolytic reactions.[3] These were connected to the breakdown of the polymers and accumulation of degradation products. Pennings et al. studied the cytotoxicity of poly(96L-/4D-lactide) and its accumulated degradation products.[4] Their results suggested that the cytotoxicity of PLA96 is related to the pH and possibly to the osmolarity of the extracts, which depends on the variations in the amount of lactic acid and lactic acid oligomers. Methods have been developed to determine or predict the microclimate pH in PLGA microspheres and films. pH-sensitive fluorescent dyes were trapped in PLGA microspheres to visualize, by confocal fluorescence microscopy, the spatial and temporal distribution of pH within the degrading microspheres.[5] Very acidic environments with pHs as low as 1.5 were sometimes detected. An equilibrium mathematical model that accurately predicts microclimate pH in thin biodegradable poly(lactide-*co*-glycolide) films has also been described.[6] In addition to causing negative effects in the body, acidic degradation products can accelerate the hydrolysis rate and influence the stability of pH-sensitive drugs.

Degradation properties are of crucial importance for the successful use of biomedical materials. Copolymerization has for many years been used to adjust the properties and degradation times for different applications. Different additives have also been tested to modify the hydrolysis rate.[7] Recent developments in polymer synthesis have increased the possibility to design chemical and macromolecular structures to prepare new

(1) Ignatius, A. A.; Claes, L. E. *Biomaterials* **1996**, *17*, 831−839.
(2) Suganuma, J.; Alexander, H. *J. Appl. Biomater.* **1993**, *4*, 13−27.
(3) Bostman, O.; Hirvensalo, E.; Makinen, J.; Rokkanen, P. *J. Bone Joint Surg.* **1990**, *72-B*, 592−596.
(4) Cordewener, F. W.; van Geffen, M. F.; Joziasse, C. A. P.; Schmitz, J. P.; Bos, R. R. M.; Rozema, F. R.; Pennings, A. J. *Biomaterials* **2000**, *21*, 2433−2442.
(5) Fu, K.; Pack, D. W.; Klibanov, A. M.; Langer, R. *Pharm. Res.* **2000**, *17*, 100−106.
(6) Ding, A. G.; Shenderova, A.; Schwendeman, S. P. *J. Am. Chem. Soc.* **2006**, *128*, 5384−5390.
(7) Renouf-Glauser, A. C.; Rose, J.; Farrar, D. F.; Cameron, R. E. *Biomacromolecules* **2006**, *7*, 612−617.

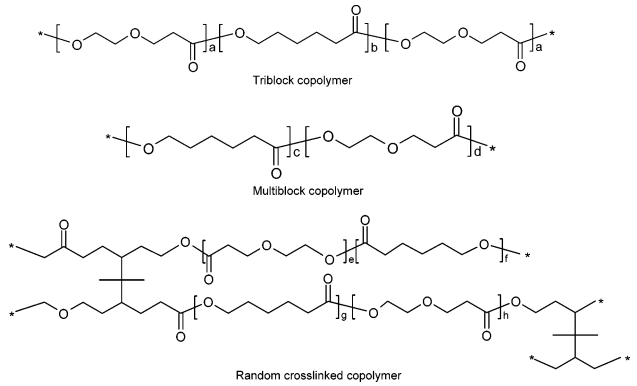
10.1021/ja0702871 CCC: $37.00

specialized polymers with superior functionalities and properties. As an example our group has synthesized different copolymers from caprolactone (CL), lactide (LA), and 1,5-dioxepan-2-one (DXO), including triblock[8] and multiblock[9] copolymers, porous structures,[10] star-shaped polymers,[11] and cross-linked networks.[12] The degradation of aliphatic polyesters has been widely studied, and the interest has only accelerated during the last years.[13,14] In several studies we have also investigated the hydrolytic degradation of different CL, LA, and DXO homopolymers and random copolymers.[15−17] Lately, several papers have compared the hydrolysis rates of block and random copolymers.[18−20] In most cases the random copolymers exhibited the fastest hydrolysis rate. While much is known of the factors affecting the degradation and degradation rate, less attention has been paid to the low-molecular weight products formed during hydrolysis and especially to finding ways to control the release rate of acidic degradation products, a crucial parameter in biomedical applications. To avoid any negative effects, neutralization of acidic degradation products by addition of basic salts[21] or blending with polymers that form basic degradation products[22] has been suggested. For the present study three CL/DXO copolymers with the same composition, but different macromolecular structures, i.e., DXO/CL/DXO triblock, CL/DXO multiblock, and random cross-linked CL/DXO copolymers were synthesized, and their hydrolytic degradation as well as the release rate of acidic degradation products was investigated. Our hypothesis was that the release rate of acidic degradation products can be controlled through macromolecular design, i.e., different distribution of weak linkages. Ideally the degradation mechanism could be tailored to independently adjust the degradation rate and release rate of water-soluble products. Through controlled release of acidic degradation products inflammatory responses caused by biomaterials could then be avoided or diminished.

Results and Discussion

Three different caprolactone/1,5-dioxepan-2-one (CL/DXO) copolymers, triblock, multiblock, and random cross-linked as well as poly(ϵ-caprolactone) (PCL) homopolymer were hydrolyzed in phosphate buffer solution for up to 364 days. After different time periods degradation products, mass loss, water absorption, molecular weight changes, and changes in thermal properties were determined to evaluate macromolecular engineering as a tool to tune the degradation rate and release profile. The structures of the copolymers are shown in Scheme 1. Copolymer compositions, sequence lengths for multiblock copolymer, and the original molecular weights are given in Table 1.

Scheme 1. Chemical Structures of (a) DXO/CL/DXO Triblock Copolymer, (b) CL/DXO Multiblock Copolymer, and (c) Random Cross-Linked CL/DXO Copolymer



Degradation Products. Monomeric hydroxyacids (6-hydroxyhexanoic acid (HHA) and 3-(2-hydroxyethoxy)propanoic acid (HPA)) were extracted from the buffer solution after the hydrolytic degradation of the studied CL/DXO copolymers. 6-Hydroxyhexanoic acid originates from the hydrolysis of CL units and 3-(2-hydroxyethoxy)propanoic acid from the hydrolysis of DXO units in the copolymers. Parts a, b, and c of Figure 1 show the relative amounts of 6-hydroxyhexanoic acid and 3-(2-hydroxyethoxy)propanoic acid formed during hydrolysis of the three CL/DXO copolymers, and part d of Figure 1 shows the relative amount of 6-hydroxyhexanoic acid formed during the hydrolysis of PCL homopolymer. The monomer ratios for the copolymers were 60 mol % CL and 40 mol % DXO; however, the arrangement of the monomers varied from triblock to multiblock and random cross-linked copolymer. Comparison of a, b, and c of Figure 1 shows that, even though the CL/DXO ratio in the copolymers was the same, the macromolecular design had large influence on both the total amount of monomeric products and on the release rate of the individual acids.

The largest amount of 3-(2-hydroxyethoxy)propanoic acid was released during the hydrolysis of triblock copolymer (Figure 1a). Depending on the hydrolysis time, 2 to 3 times more 3-(2-hydroxyethoxy)propanoic acid was formed during the hydrolysis of triblock copolymer compared to the hydrolysis of multiblock and random copolymers. Amorphous, hydrophilic PDXO is more susceptible to hydrolysis than semicrystalline, hydrophobic PCL. The long DXO blocks in the triblock copolymer are also hydrophilic, amorphous regions, which makes them more vulnerable to hydrolysis compared to the short DXO blocks in the multiblock copolymer or the randomly distributed DXO units in the random copolymer. Triblock and multiblock copolymers had similar degrees of crystallinity. However, "the distribution of the crystallinity" differed. In the triblock copolymer CL blocks are crystalline, and DXO blocks are amorphous regions. In the multiblock copolymer it is probable that some of the DXO units are incorporated into the CL crystals which makes them less accessible to water and thus less susceptible to hydrolysis.

(8) Ryner, M.; Albertsson, A.-C. *Biomacromolecules* **2002**, *3*, 601−608.
(9) Andronova, N.; Finne, A.; Albertsson, A.-C. *J. Polym. Sci., Part A: Polym. Chem.* **2003**, *41*, 2412−2423.
(10) Odelius, K.; Plikk, P.; Albertsson, A.-C. *Biomacromolecules* **2005**, *6*, 2718−2725.
(11) Odelius, K.; Finne, A.; Albertsson, A.-C. *J. Polym. Sci., Part A: Polym. Chem.* **2006**, *44*, 596−605.
(12) Andronova, N.; Srivastava, R. K.; Albertsson, A.-C. *Polymer* **2005**, *46*, 6746−6755.
(13) Hakkarainen, M. *Adv. Polym. Sci.* **2002**, *157*, 113−138.
(14) Vert, M. *e-Polymers* **2005**, *8*, 1−10.
(15) Karlsson, S.; Hakkarainen, M.; Albertsson, A.-C. *J. Chromatogr., A* **1994**, *688*, 251−259.
(16) Hakkarainen, M.; Albertsson, A.-C.; Karlsson, S. *Polym. Degrad. Stab.* **1996**, *52*, 283−291.
(17) Hakkarainen, M.; Karlsson, S.; Albertsson, A.-C. *Polymer* **1999**, *41*, 2331−2338.
(18) Jeon, O.; Lee, S.-H.; Kim, S. H.; Lee, Y. M.; Kim, Y. H. *Macromolecules* **2003**, *36*, 5585−5592.
(19) Li, S.; Dobrzynski, P.; Kasperczyk, J.; Bero, M.; Braud, C.; Vert, M. *Biomacromolecules* **2005**, *6*, 489−497.
(20) Zurita, R.; Puiggali, J.; Franco, L.; Rodriguez-Galan, A. *J. Polym. Sci., Part A: Polym. Chem.* **2006**, *44*, 993−1013.
(21) Agrawal, C. M.; Athanasiou, K. A. *J. Biomed. Mater. Res.* **1997**, *38*, 105−114.
(22) Ambrosio, A. M. A.; Allcock, H. R.; Katti, D. S.; Laurencin, C. T. *Biomaterials* **2002**, *23*, 1667−1672.

Table 1. Copolymer Compositions, Sequence Lengths for the Multiblock Copolymer, and the Original Molecular Weights and Polydispersities

	polymer composition [%]		sequence length[b]			
polymer name	CL	DXO	L_{CL}	L_{DXO}	M_n[c] [g/mol]	PDI[c]
multiblock	63 ± 0.2[a]	37 ± 0.2[a]	6.0 ± 0.3	3.1 ± 0.1	42000 ± 6400	1.65 ± 0.11
triblock	61 ± 0.3[a]	39 ± 0.3[a]	–	–	47200 ± 400	1.51 ± 0.05
homo	100	–	–	–	30000 ± 600	1.45 ± 0.01
network	60[d]	40[d]	–	–		

[a] Determined by ^{1}H NMR using $\delta_{CL} = 2.30$ ppm and $\delta_{DXO} = 3.75$ ppm. [b] Determined using ^{13}C NMR. [c] Determined using DMF SEC calibrated with narrow molecular weight polystyrene standards. [d] Monomer feed composition.

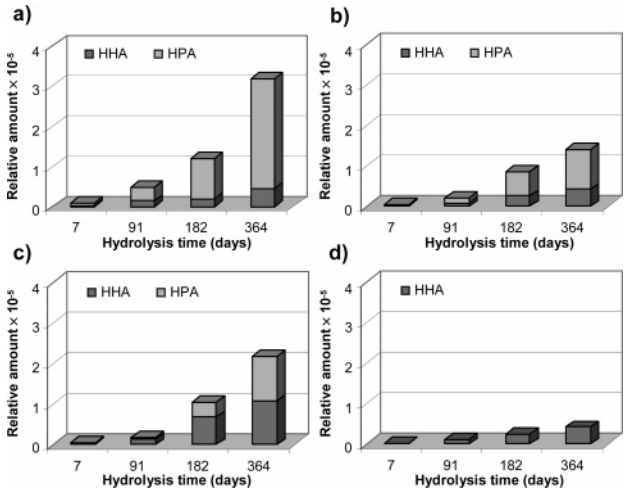


Figure 1. Relative amounts of 6-hydroxyhexanoic acid (HHA) and 3-(2-hydroxyethoxy)propanoic acid (HPA) formed during hydrolysis of (a) DXO/CL/DXO triblock copolymer, (b) CL/DXO multiblock copolymer, (c) random cross-linked CL/DXO copolymer, and (d) PCL homopolymer. The polymers were hydrolyzed for different times in phosphate buffer pH 7.4 and 37 °C. After the predetermined hydrolysis times the monomeric degradation products were extracted by solid-phase extraction and analyzed by GC−MS.

This incorporation of DXO units into the CL crystals has earlier been shown for random CL/DXO copolymers.[23]

The amount of 3-(2-hydroxyethoxy)propanoic acid released from multiblock and random copolymers was approximately the same. However, twice as much 6-hydrohexanoic acid was released from the random copolymer, compared to that from the triblock and multiblock copolymers. After 364 days the amount of 3-(2-hydroxyethoxy)propanoic acid released from triblock copolymer was almost 6 times higher than the amount of 6-hydroxyhexanoic acid, but approximately the same amounts of 3-(2-hydroxyethoxy)propanoic and 6-hydroxyhexanoic acids were released from the random copolymer. This is explained by two contributing factors. The CL blocks in the tri- and multiblock copolymers have a relatively high degree of crystallinity, while the random cross-linked DXO/CL copolymer is amorphous material, which increases the degradation rate of CL units. In addition, as the CL units are randomly distributed, the release of 6-hydroxyhexanoic acid is facilitated by the hydrolysis of the more easily hydrolyzed DXO units. The amount of 6-hydroxyhexanoic acid released from the PCL homopolymer was similar to the amount released from the tri- and multiblock copolymers; however, if related to the CL content, the hydrolysis rate of PCL homopolymer is considerably lower than the hydrolysis rate of CL blocks in the tri- and multiblock copolymers. This is explained by the higher overall crystallinity and the lower hydrophilicity of the PCL homopolymer. The hydrolysis of DXO units also renders the hydrolysis of CL blocks.

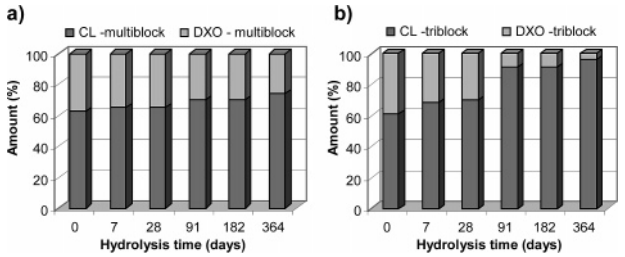


Figure 2. Composition of (a) CL/DXO multiblock and (b) DXO/CL/DXO triblock copolymers after different hydrolysis times as determined by ^{1}H NMR by comparing the peak intensities of the comonomers, CL and DXO. The copolymers were hydrolyzed in phosphate buffer, pH 7.4, and 37 °C.

After 364 days the total amount of acidic monomeric hydrolysis products released from the triblock copolymer was almost 3 times higher compared to that from the multiblock copolymer, which had the lowest release rate of monomeric products of the three copolymers. Almost twice as much monomeric product was released from the random copolymer compared to the multiblock copolymer.

Hydrolysis-Induced Changes in the Copolymer Composition. Figure 2 shows how the composition of multiblock and triblock copolymers changes during the hydrolysis as determined by NMR. In the triblock copolymer the two DXO blocks were almost totally hydrolyzed, leaving behind the CL block, which was much more resistant toward hydrolysis. After 91 days the DXO content in the triblock copolymer had decreased from 40% to 9%. The composition of the multiblock copolymer remained much more constant, and after 364 days the DXO content was still 26% compared to the DXO content in the triblock copolymer which had decreased to 4%. These results correlate well with the GC−MS results, which also showed that DXO blocks in the triblock copolymer were hydrolyzed, leading to formation of large amount of 3-(2-hydroxyethoxy)propanoic acid. Similar results have been shown for other block copolymers, i.e. valerolactone blocks were preferentially hydrolyzed in caprolactone/valerolactone copolymers.[24]

Mass Loss, Water Absorption, and Molecular Weight Changes. Figure 3 shows the mass loss as a function of hydrolysis time. In accordance with GC−MS results, mass loss was highest for the triblock copolymer and lowest for the PCL homopolymer. The mass losses for multiblock and random copolymers were rather similar. Figure 4 presents a comparison of mass loss and relative amounts of monomeric degradation products released from the different copolymers after 182 days

(23) Albertsson, A.-C.; Gruvegaard, M. *Polymer* **1995**, *36*, 1009−1016.

(24) Fay, F.; Linossier, I.; Langlois, V.; Renard, E.; Vallee-Rehel, K. *Biomacromolecules* **2006**, *7*, 851−857.

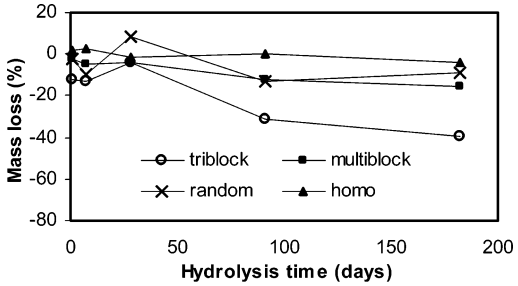


Figure 3. Mass loss for DXO/CL/DXO triblock copolymer, CL/DXO multiblock copolymer, random cross-linked CL/DXO copolymer and PCL homopolymer as a function of hydrolysis time. The hydrolysis was performed in phosphate buffer, pH 7.4, and 37 °C.

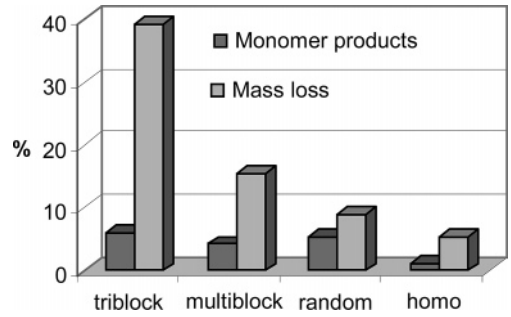


Figure 4. Comparison of mass loss and relative amount of monomeric degradation products released from DXO/CL/DXO triblock copolymer, CL/DXO multiblock copolymer, random cross-linked CL/DXO copolymer, and PCL homopolymer after 182 days of hydrolysis in phosphate buffer, pH 7.4, and 37 °C. The monomeric products were extracted from the buffer solution with solid-phase extraction and analyzed by GC−MS.

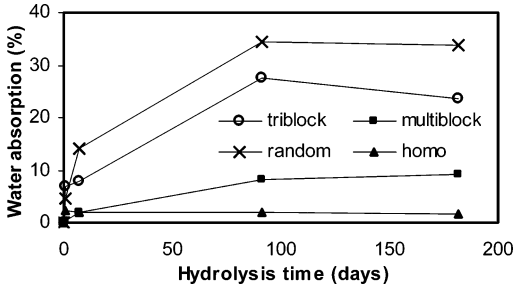


Figure 5. Water uptake of DXO/CL/DXO triblock copolymer, CL/DXO multiblock copolymer, random cross-linked CL/DXO copolymer, and PCL homopolymer after different hydrolysis times in phosphate buffer, pH 7.4, and 37 °C.

of hydrolysis. The triblock copolymer had the highest degradation rate as measured by mass loss and amount of monomeric hydrolysis products. However, relative to the mass loss, the largest amount of monomeric hydrolysis products was released from the random cross-linked polymer, and the lowest amount was from the triblock copolymer. This means that the largest amount of water-soluble oligomers was released from triblock and the lowest amount was from random cross-linked copolymers. The cross-linking in the random copolymer inhibited the release of low-molecular weight products, leading to lower mass loss. DXO oligomers were also more hydrophilic than CL oligomers, which additionally facilitated the release of water-soluble oligomers from the triblock copolymer.

Figure 5 shows that generally the water uptake was in good correlation with the mass loss. An exception was the random cross-linked copolymer, which absorbed the largest amount of water. It was the only amorphous copolymer, which makes it easier for water to migrate into the material. Due to the cross-

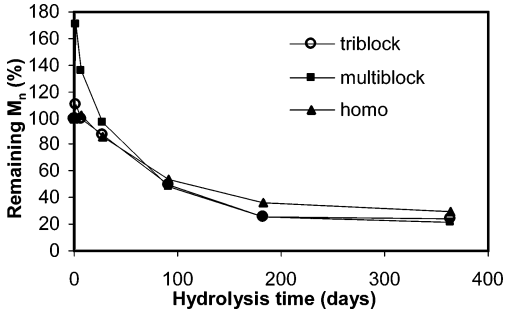


Figure 6. Molecular weight changes of DXO/CL/DXO triblock copolymer, CL/DXO multiblock copolymer, and PCL homopolymer caused by hydrolysis in phosphate buffer, pH 7.4, and 37 °C. The molecular weights were measured by SEC using *N*,*N*-dimethylformamide as a solvent.

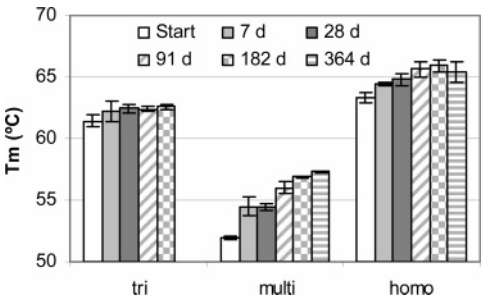


Figure 7. Melting temperatures for DXO/CL/DXO triblock copolymer, CL/DXO multiblock copolymer, and PCL homopolymer determined from the first DSC heating scan after different hydrolysis times in phosphate buffer, pH 7.4, and 37 °C.

linking, a larger number of chain scissions was needed before water soluble products were formed, which can explain the lower mass loss in relation to water uptake. This was in agreement with the other results showing that mainly monomeric products were released from the random copolymer compared to those from multiblock and triblock copolymers, which released also large amounts of oligomers. As expected the lowest amount of water was absorbed by PCL homopolymer, which has the highest degree of crystallinity and is also the most hydrophobic of the studied polymers.

Figure 6 shows that even though the release rate of acidic degradation products and the mass loss were much higher for the triblock copolymer, the molecular weight changes were rather similar the triblock copolymer, multiblock copolymer and PCL homopolymer. The molecular weight for the random copolymer could not be measured as it was not soluble due to the cross-linking. The higher mass loss for triblock copolymer in relation to the molecular weight loss can be related to the chain scissions occurring mainly in the DXO blocks. This leads to faster formation of water-soluble degradation products and slower reduction in molecular weight compared to the more random chain scission in multiblock copolymer and PCL homopolymer.

Thermal Properties. The thermal properties of the triblock, multiblock, and PCL homopolymer were evaluated by DSC, and the effect of hydrolysis on melting point (T_m) (Figure 7) and heat of fusion (ΔH_f) (Table 2) was determined. The random copolymer was amorphous and thus did not have a melting point. The initial degrees of crystallinity (w_c) for tri- and multiblock copolymers as determined from the first heating scan were almost equal, showing that when these copolymers are given time they crystallize to almost the same extent. PDXO is

Table 2. Degree of Crystallinity after Different Hydrolysis[a] Times Determined from the First Heating Scan of DSC

	w_c[b]				
polymer name	start	7 d	28 d	91 d	364 d
multiblock	49 ± 0.7	44 ± 1.2	46 ± 0.7	49 ± 3.1	68 ± 1.9
triblock	45 ± 0.3	49 ± 1.3	51 ± 0.4	61 ± 2.8	ND
homo	78 ± 0.2	72 ± 1.4	75 ± 3.0	78 ± 0.2	85

[a] The polymers were hydrolyzed in phosphate buffer, pH 7.4, and 37 °C. [b] Determined from the first heating scan.

a completely amorphous polymer.[25] However, it has been shown that DXO can be incorporated in the crystal domains of poly(DXO-*co*-CL) copolymers, and it has been suggested that short DXO segments probably act as nucleation points in the copolymer.[23] This multitude of nucleation points in the multiblock copolymer led to a faster crystallization rate and formation of a large number of smaller crystals, which also explains the lower T_m for the multiblock copolymer compared to those of the triblock copolymer and PCL homopolymer, Figure 7.

As seen in Table 2, the degree of crystallinity, w_c, increases during the hydrolysis. It is a well-known phenomenon that the amorphous regions are more susceptible to hydrolysis which leads to increasing w_c during hydrolysis of semicrystalline polyesters. The shorter chains formed during the hydrolysis also have higher mobility, allowing for reorientation of the crystalline phase, which increases w_c. The increase in T_m seen in Figure 7 is explained by continued crystallization, where the crystalline thickness increases during hydrolysis.[26]

(25) Mathisen, T.; Masus, K.; Albertsson, A.-C. *Macromolecules* **1989**, *22*, 3842−3846.
(26) Tsuji, H.; Ikada, Y. *J. Appl. Polym. Sci.* **1998**, *67*, 405−415.

Conclusions

Degradation rate, the composition of acidic degradation products, and their release rate were controllable through macromolecular design, which confirmed our original hypothesis. The release rate and pattern of acidic degradation products were tunable by different distributions of the more hydrolysis-susceptible DXO linkages in the copolymers. The largest amounts of both monomeric hydrolysis products and water-soluble oligomers were released from the triblock copolymer due to the susceptibility of the long hydrophilic DXO blocks toward hydrolysis. The highest amount of 6-hydroxyhexanoic acid was released from the random copolymer where the release of 6-hydroxyhexanoic acid was facilitated by the hydrolysis of the randomly distributed DXO linkages and by the reduced crystallinity caused by the cross-linking. Cross-linking also affected the degradation product pattern by causing a shift from water-soluble oligomers toward the monomeric hydroxyacids. Macromolecular engineering of biomaterials proves to be a valuable tool for tuning the release rate of acidic degradation products in order to prevent the formation of acidic microclimates and to reduce the risk for inflammatory reactions in the body.

Acknowledgment. We gratefully acknowledge The Swedish Foundation for Strategic Research (Grant no. A302:139) for their financial support of this work.

Supporting Information Available: Experimental details (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

JA0702871