BIODEGRADABLE POLY(ESTERS) AND THE DELIVERY OF BIOACTIVE AGENTS

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BACKGROUND

Biodegradable polymers have a variety of potential biomedical applications in addition to their current use as suture material [1]. Among the most important of these potential applications is their use in the controlled release of therapeutic agents [2]. The biodegradable polymers used in controlled release applications are primarily insoluble polymers which undergo chemical hydrolysis to soluble monomeric or oligomeric units [2]. These polymers have both advantages and disadvantages when compared to non-degradable polymers used for controlled delivery. The major advantage is that biodegradable polymers do not require removal after drug delivery is complete. In addition, adverse tissue reactions from implanted polymer are ameliorated as the polymer degrades [3]. However, because these polymers degrade with time, their removal is often difficult, as when a change in therapy is required before the entire course of therapy is complete. Also, because of their degradation, toxicology must be performed on both the polymer and all decomposition products as well as their metabolites.

Recently, there has been much interest in developing new methods to deliver both vaccines and adjuvants. In many instances delivery of an immunogen or accompanying adjuvant must be delayed or sustained over a prolonged period of time. Such sustained delivery of immunogen might result in a heightened immune response eliminating the need for boosters. Appropriate presentation of the vaccine to the immune system may also be required, particularly for highly purified antigens prepared by recombinant technology.

Poly(esters) were chosen for a study as a vaccine delivery vehicle because they are currently widely used in suture materials and both the polymer and their decomposition products have demonstrated low toxicity. Poly(esters) generally degrade in four major stages: (1) Polymer hydration, (2) Strength loss caused by breakage of backbone bonds in polymer, (3) Loss of mass integrity, and (4) Solubilization [2]. Since matrix biodegradation generally involves bulk erosion, drug release generally takes place prior to matrix biodegradation under diffusion-control. This has created problems in the delivery of drugs where zero-order release kinetics is often desirable [2,4]. In the application of these polymers to vaccine delivery, precise control of release kinetics may not be as important as delayed or sustained release. In addition, the need to remove a vaccine-containing device before an entire dose is delivered is not anticipated, eliminating the difficulty of recovering biodegradable microspheres [5].

This research focuses on biodegradable poly(esters) as potential carriers of recombinant proteins for vaccine use. Poly(esters) used medically as suture material [1] were first used in a drug delivery applications for the release of narcotic antagonists from poly(lactide) [4]. Initial experiments confirm previous studies that a wide variety of matrix life-times can be obtained by altering the monomer composition of the poly(ester) and its molecular weight [6]. Studies on the change in properties of poly(lactide:glycolide, 50:50) during biodegradation have also been performed. Finally, poly(ester) microspheres containing protein have been prepared and in vitro protein release has been examined.

MATERIAL AND METHODS

Poly(lactide:glycolide, 50:50 and 85:15) (Medisorb, bioresorable polymers) were from Dupont. Poly(3-hydroxybutyrate-co-3-hydoxyvalorate, 93:7 and 80:20) and poly(3-hyroxybutyrate) were from Goodfellow. Poly(esters) including poly(L-lactide) MW 50,000, 100,000 and 200,000; Poly(D,L-lactide-co-glycolide, 80:20), poly(3(-) hydroxybutyrate) MW 50,000, and poly(3-hydroxybutyrate-co-3-hydroxyvalerate, 80:20) were from Polysciences Inc. Poly(anhydride), purified bis(p-carboxyphenoxy) propane/sebacic acid copolymer (PCPP-SA 20:80) of MW 30,000 [7] was a gift from Drs. R. Langer and E. Ron of the Department of Chemical Engineering, MIT. Polymers were

used either directly as powders, as particles or were spray-dried from a 1-10 (w/v)% solution in methylene chloride at 25°C using a Yamato Pulvis Min-Spray GA-32 spray-drier. The particles formed by spray drying were measured by scanning electron microscopy.

Polymer molecular weight was determined by dissolving the polymer at 0.5(w/v)% in either methylene chloride or tetrahydrofuran and injecting 50 µl onto a Ultrastyragel® (mixed bed) column equilibrated with the same solvent. A flow rate of 1 ml/min. at ambient temperature with refractive index detection was used. The column was calibrated using polystyrene molecular weight standards (Polyscience). Viscosity was measured in methylene chloride at 25°C using a Cannon-Ubbelohde capillary viscometer. Proton NMR were obtained on polymer in deuterochloroform with a TMS internal standard and on hydrolysis products in deuterium oxide with DSS internal standard on a Bruker WM 360 NMR spectrometer. Differential scanning calorimetry (DSC) thermograms were obtained on a Perkin Elmer 7 series thermal analysis system.

Biodegradation studies were conducted by placing polymer (200 mg) into 2.5 ml of phosphate buffered saline (PBS 150, MM anhydrous sodium phosphate (dibasic) and 0.9 (w/v)% sodium chloride at pH 7.2), and shaking at 37°C. Biodegradation studies at 85°C were performed in an oil bath without agitation. Soluble products were obtained by periodically removing all the PBS from the vessel containing polymer and replacing it with the same volume of fresh PBS. Analysis for soluble biodegradation products was conducted with a Dionex QIC ion-chromatography system on an HPICE-AS1 column eluted with octanesulfonic acid (2 mM) for butyrate, valorate, and sebacic acid or an HPICE-AS5 column eluted with heptafluorobutyric acid (1.6 mM) for lactate and glycolate. A post-column AMMS-ICE membrane suppressor column run with 10 mM tetra-n-butylamnonium hydroxide at a flow rate of 1 ml/min was used prior to ion-detection by conductance (100 µS full scale) [7]. The polymer residue was recovered, washed with water and dried under under vacuum. The weight of the residue was recorded and it was then dissolved in methylene chloride for analysis by viscometry and gel permeation chromatography.

Protein release studies were conducted using poly(lactide:glycolide, 50:50) microspheres (5 micron) containing 5% BSA. These were prepared by suspending spray dried 50 mg of BSA (5 micron particles) in methylene chloride (50 ml) containing 1000 mg of dissolved poly(ester) and then spray drying. The microspheres (200 mg) were placed in 2.5 ml of PBS at 37°C. No surfactant was added to the release medium which led to beads which aggregated during the release/hydrolysis study. Every 24 hours the PBS was completely removed and the protein content was measured using Bradford's method [8]. Fresh PBS (2.5 ml) was then added to replenish that which had been removed.

RESULTS AND DISCUSSION

A number of commercially available poly(esters) of different molecular weights and composed of various hydroxyacid monomers including lactide, glycolide, ß-hydroxybutyrate and ß-hydroxyvalerate, together with a single poly(anhydride) were studied (Table 1). The molecular weight of each polymer was determined in a suitable solvent by gel permeation chromatography. The poly(esters) chosen for study had molecular weights ranging from less than 10,000 to over 400,000 Da (Table 1). Preliminary hydrolysis studies on these polymers, performed in PBS, demonstrated a range of hydrolysis rates. Hydrophobic polymers such as poly(ß-hydroxbutuyrate: ß-hydroxyvalerate) **(10,11)** hydrolyzed slowly over many months. Even at an elevated temperature of 85°C over half of this polymer remained after 100 days. Poly(lactide) polymers **(1,2&3)** showed increasing time to half-erosion, ranging from 2-50 days at 85°C with increasing polymer molecular weight (Table 1). Poly(lactide:glycolide) polymers**(4-8)** showed increasing hydrolysis rates as the monomer ratios approached 50:50. The hydrolysis rate also increased with increasing temperature. Poly(anhydride) hydrolyzed more rapidly than did any of the poly(esters) due to the inherent hydrolyic instability of the anhydride linkage [2,9,10].

Poly(lactide:glycolide, 50:50) was chosen for further study because it demonstrated a sufficiently rapid rate of hydrolysis to be useful for certain vaccine delivery applications. Proton NMR confirmed the composition of this polymer to be 50:50 and its glass transition (Tg) was determined by DSC to be 43°C. During the first 5 hours of hydrolysis in PBS at 85°C, little

Table 1

Properties of Various Biodegradable Polymers

Polymer	Monomer[a] Composition	Molecular Weight Reported kDa	Molecular Weight Determined kDa	Time to half-erosion[e] in PBS (days) 37°C	Time to half-erosion[e] in PBS (days) 85°C
1	1.0L	50	nd[d]	nd	2
2	1.0L	100	nd	nd	11
3	1.0L	200	nd	nd	50
4	0.9L,0.1G	-	10[b]	nd	nd
5	0.85L,0.15G	60-100	63[b]	100	nd
6	0.8L,0.2G	-	8[b], 18[c]	nd	1
7	0.7L,0.3G	-	4[b], 14[c]	nd	0.1
8	0.5L,0.5G	60-100	60, 60	40	1
9	1.0B	50	nd	nd	110
10	0.93B,0.07V	-	nd	nd	150
11	0.8B,0.2V	-	400[b]	nd	130
12	0.2PCPP,0.8SA	43 [10]	3[b]	20	0.2

a. Monomers (preceded by mole fraction) are: L, lactide; G, glycolide, B, ß-hydroxybutyrate; V, ß-hydroxyvalerate; PCPP, bis(p-carboxyphenoxy) propane; and SA, sebacic acid.
b. Molecular weight determined by GPC in methylene chloride using polystyrene molecular weight standards.
c. Molecular weight determined by GPC in tetrahydrofuran using polystyrene molecular weight standards.
d. nd, not determined.
e. Calculated by measuring the cumulative monomer released using ion-chromatography with conductivity detection.

or no mass loss was observed and the viscosity of the polymer residue only decreased slightly (Table 2). The number average molecular weight decreased from 60,300 to 12,600 over the same 5 hour period. After 5 hours, further decrease in molecular weight was accompanied by rapid loss of in the mass of polymer residue (Table 2). The data presented in Table 2 is entirely consistent with the mechanism of poly(ester) degradation presented earlier in this paper. The formation of both soluble monomer products were also measured over time (Figure 1). Glycolic acid was formed initially at a higher rate than lactic acid. After residual mass loss had begun to take place, a sample of soluble product was examined by NMR. In addition to the major monomeric products, evidence for the presence of soluble oligomeric products was seen in the form of additional minor signals at 1.41 ppm and 1.50 ppm which disappeared after the sample sat overnight in deuterium oxide.

Release of protein entrapped in poly(lactide:glycolide, 50:50) microspheres was also examined in an *in vitro* study in PBS at 37°C. Microspheres (5 micron) were prepared which contained 5 w/w% BSA. Table 3 shows the results of a release study conducted over a period of one week. Virtually all (> 90%) of the BSA was released over the short time frame of this study. Based on hydrolysis studies conducted on spray-dried poly(lactide: glycolide, 50:50) in the absence of loaded protein, it is anticipated that less than 10% of the matrix would have solubilized during 7 days at 37°C. Most of the release takes place in day 1 possibly suggesting the incomplete entrapment of protein by polymer matrix using spray-drying techniques. Further efforts, including new approaches in formulation, will be required to decrease the rapid rate of release of protein from poly(ester) microspheres.

Table 2

Change in the Properties of Poly(lactide:glycolide 50:50) During Biodegradation

Hydrolysis Time [a] (hr)	Residual [b] Mass(mg)	Viscosity [c] [η](dl/g)	Molecular Weight [d]
0	250	0.34	60,300
1	247	0.31	50,000
3	255	0.23	17,800
5	252	0.21	12,600
10	240	0.06	2,000
16	183	0.04	800
24	74	--	700
48	41	--	--
72	7	--	--
96	2	--	--

a. Poly(lactide:glycolide, 50:50) pellets (250 mg) were suspended in 5ml PBS at 85°C.
b. Measure by recovering polymer, washing with water, drying and weighing.
c. Viscosity of recovered, dried polymer measured using Cannon-Ubbelohde viscometer in methylene chloride at 25°C.
d. Molecular weight measured in methylene chloride using polystyrene molecular weight standards.

Figure 1

The hydrolysis time in days is plotted against the accumulated millimolar concentration of glycolate and lactate products. The inset shows the ratio of glycolate to lactate formed over the same time-frame.

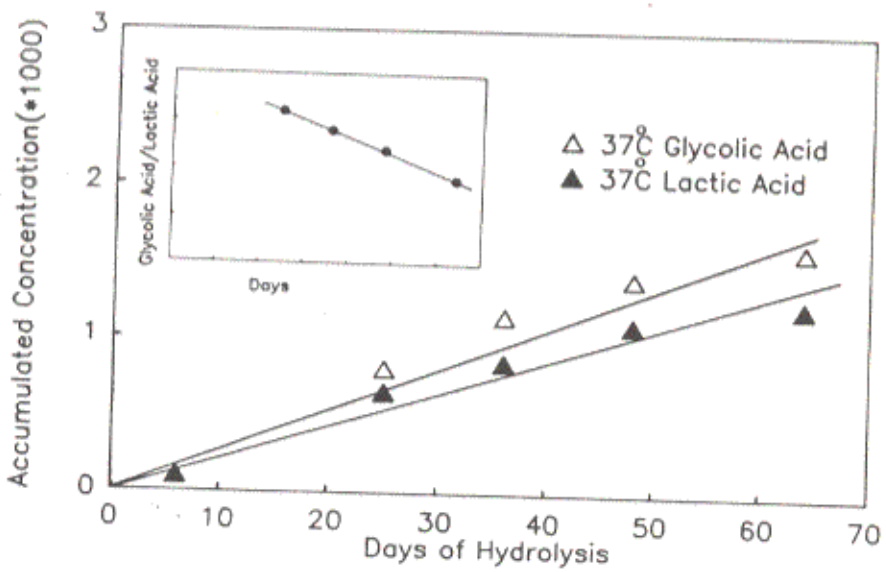

Table 3

Release of Entrapped Protein from
Poly(lactide:glycolide, 50:50) Microspheres

Time (days)	% of Soluble BSA Measured	% BSA Remaining in Polymer Calculated
1	3.60	28
2	0.60	16
3	0.12	14
4	0.12	11
5	0.10	9
6	0.03	8.6
7	0.02	8.4

Total = 4.59% released / 5.00% loaded = 91.6% recovery of BSA

ACKNOWLEDGEMENT

The authors are grateful for Salsbury Laboratories Inc. for generous support of this research.

REFERENCES

1. Anon. Chem. Ind., 905 (1970).

2. Linhardt, R.J., Biodegradable Polymers for the Controlled Release of Drugs in Controlled Release of Drugs: Polymer and Aggregate Systems, M. Rosoff, ed., VCH Publishers, New York, Chapter 2, pp. 53-95 (1989)

3. Yoles, S. and Sartori, M.F., Drug Delivery Systems, 84 (1980).

4. Yokes, S., Eldridge, J.E., and Woodland, J.H.R., Polymer News, 1, 9 (1971).

5. Thies, C. Crit. Rev. Biomed. Eng., 8, 335 (1985).

6. Pitt, C.G. and Schindler, A. in Biodegradable and Drug Delivery Systems for Contraceptives, E.S.E. Hafe, and W.A. Van Os, Eds., MTP Press, Lancaster, England, Vol. 1 (1980).

7. Linhardt, R.J., Flanagan, D.R., Schmitt, E., Wang, H.T., Polymer Prints, 30, 464 (1989).

8. Bradford, M. Anal. Biochem., 72, 248 (1976).

9. Rosen, H.B., Chang, J., Wnek, G.E., Linhardt, R.J., and Langer, R., Biomaterials, 4, 131 (1983).

10. Leong, K., Simonte, V., and Langer, R., Macromolecules, 20, 705 (1987).