Polymer Bulletin 42, 419–424 (1999)

Polymer Bulletin

Enzyme-catalyzed degradation of aliphatic polycarbonates prepared from epoxides and carbon dioxide

Ming Zhou, Masaharu Takayanagi, Yasuhiko Yoshida*, Shigeru Ishii, Hiromichi Noguchi

Department of Applied Chemistry, Faculty of Engineering, Toyo University, Kujirai, Kawagoe, Saitama 350-8585, Japan

Received: 25 January 1999/Accepted: 25 February 1999

Summary

Films of aliphatic polycarbonates prepared from epoxides and carbon dioxide were found for the first time to be degradable with a single enzyme, *Rhizopus delemar* lipase, at 37°C in an acetate buffer solution. In the degradation of polycarbonates containing oxyethylene units, ethylene glycol could be detected in the buffer solution, and was quantified by GLC after conversion to its diacetate. The weight loss values after 168 h were in close agreement with those calculated from the yields of ethylene glycol. Under the same conditions the polycarbonates were degraded much more reluctantly than poly(butylene succinate), an aliphatic polyester. *Rhizopus arrhizus* lipase was not effective for the degradation of any of these polymers.

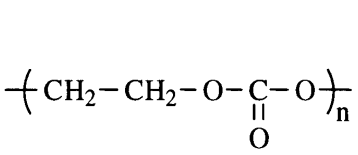
Introduction

Synthetic commodity polymers are in general hardly degradable in nature, causing ecological problems when wasted. Thus, there is a strong need for polymeric materials which degrade in nature without producing any harmful stuffs but exhibit excellent properties for various purposes. Taking into consideration these as well as the fact that synthesis of polymeric materials using carbon dioxide as one of raw materials is a promising technology for fixation and recyclization of CO_2, particularly the CO_2 in the environment, we have already studied on the preparation of aliphatic polycarbonates by copolymerization of epoxides with CO_2 (1), and found that the resulted copolymers could be degraded either in acidic and alkaline solutions (2) or in the peritoneal cavity of rats without causing any visible inflammation reaction (3). More recently, Nishida and Tokiwa (4) have reported on the degradation of poly(1,3-dioxolan-2-one) by microorganisms.

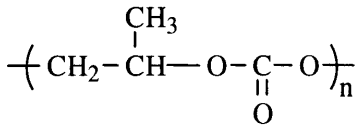
In general, biodegradation of polymeric materials *in vivo* or by microorganisms is complex, greatly depending on experimental conditions. Therefore, it is difficult to establish a genuine degradation mechanism. On the other hand, biodegradation of a polymeric material with a single enzyme permits one to investigate on the degradation in a definite manner using a small amount of sample under controlled conditions. Thus, in the present study, we have investigated on the degradation of the aliphatic polycarbonates, which were

* Corresponding author
Tel.: +81-492-39-1368; Fax: +81-492-39-1389
e-mail: yoshida@eng.toyo.ac.jp

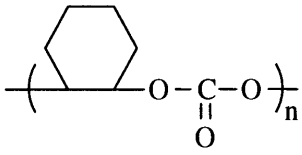
prepared from ethylene oxide (EO), propylene oxide (PO) or cyclohexene oxide (CHO) and CO_2, with enzymes which have been known to be effective for the hydrolysis of polyesters (5, 6), and found for the first time that they can be degraded by a sort of lipase. Listed below are the structures and notation of the polycarbonates prepared and poly(butylene succinate) (PBS) for the sake of comparison.



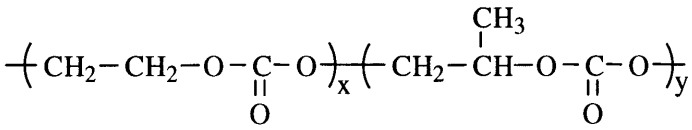
EO-CO_2



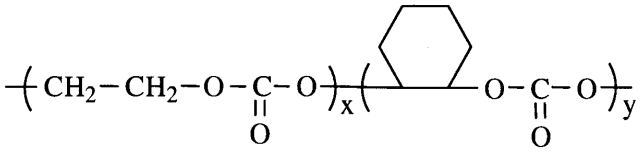
PO-CO_2



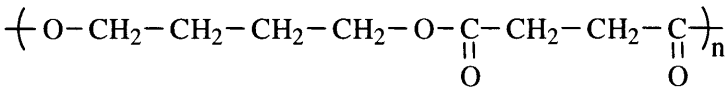
CHO-CO_2



EO-PO-CO_2



EO-CHO-CO_2



PBS

Experimental

Measurements

^{1}H NMR spectra of polycarbonates were recorded on a JEOL FX90Q Fourier Transform NMR spectrometer. The molecular weights of copolymers were measured by use of a Shodex GPC 804L column and a refractive index detector, RID-6A, on a Shimadzu C-R4A CHROMATOPAC, using polystyrene as a standard. The Tg values of the polycarbonates were determined by differential scanning calorimetry using a DSC instrument of Seiko Instruments Inc., DSC 22.

Synthesis

The aliphatic polycarbonates were prepared from epoxides and CO_2 according to the procedure of Inoue et al. (1).

Degradation

Films of the copolymers as well as of a commercial biodegradable polyester, PBS [Bionolle®; Showa Highpolymer Co., Ltd., Tokyo], were prepared from chloroform solutions by the solvent casting method. Two enzymes as purchased were employed as catalysts for hydrolysis of polymers: *Rhizopus arrhizus* lipase (Seikagaku Corporation; activity, 588,800 U/mg) and *Rhizopus delemar* lipase (Sigma Chemical Co.; activity, 600

U/mg). A small piece of a polymer film (size, ca. 10 mm x 10 mm; weight, 4-10 mg; thickness, 10-20 μm), placed in a polyethylene bag of 1 mm meshes, was immersed in 50 mL of acetate buffer solution (pH 5.6) containing a Ca^{2+} activator at 37°C for 30 min. Then, degradation reaction was started by addition of a powdery enzyme in an amount of 10,000 U/mg of copolymer or of 100 U/mg of PBS. After a given time, the polymer sample was taken out of the reaction vessel, washed thoroughly with pure water, and dried under high vacuum. In the case of *R. arrhizus* lipase, a phosphate buffer solution (0.03M, pH 7.2) was used instead of the acetate buffer solution.

For the quantification of the ethylene glycol formed by the degradation, the buffer solution after 168 h degradation was evaporated to near dryness and then treated with a large excess of acetyl chloride and pyridine to obtain ethylene glycol diacetate. The latter was quantified by GLC.

Results and discussion

Table 1 presents the reaction conditions for the preparation of epoxide-CO_2 copolymers, yields, copolymer compositions estimated by ^1H NMR, and their characterization data such as molecular weights and Tg values. The IR absorption bands at 1250 and 1750 cm^{-1}, characteristic of carbonate groups, and the area ratios of the peaks of ^1H NMR spectra of the ternary copolymers have established the alternating structures of the epoxide-CO_2 copolymers (see f_{CO_2}) and also revealed the following order of reactivity of epoxides in the copolymerization reaction: CHO > EO > PO.

Table 1 Copolymerization of carbon dioxide and epoxides [a] and characterization of the resulted polycarbonates

No.	Monomer [b]			Reaction Time (h)	Copolymer				
	M_1	M_2	(mL)		Yield (g) [c]	$\overline{Mn}/10^4$	M_2/M_1 [d]	fco_2 [e]	Tg (°C)
1	EO	-	10.0	68	1.25	0.4	-	49	2
2	PO	-	13.0	68	3.85	14.1	-	49	38
3	CHO	-	14.8	44	2.88	5.6	-	50	125
4	EO	PO	4.0/5.1	68	1.43	0.4	0.8	46	7
5	EO	CHO	4.0/7.4	66	1.68	2.5	1.2	48	16

[a] Catalyst system: $H_2O/ZnEt_2$=0.9; monomer/$ZnEt_2$=20; [$ZnEt_2$]=[H_2O]=0.807 mol/L in 1,4-dioxane; reaction temperature, 40°C. [b] Molar ratio: M_1/M_2=1/1. [c] Methanol insoluble portion. [d] Molar ratio of epoxides. [e] Percent content of oxycarbonyl unit in the copolymer.

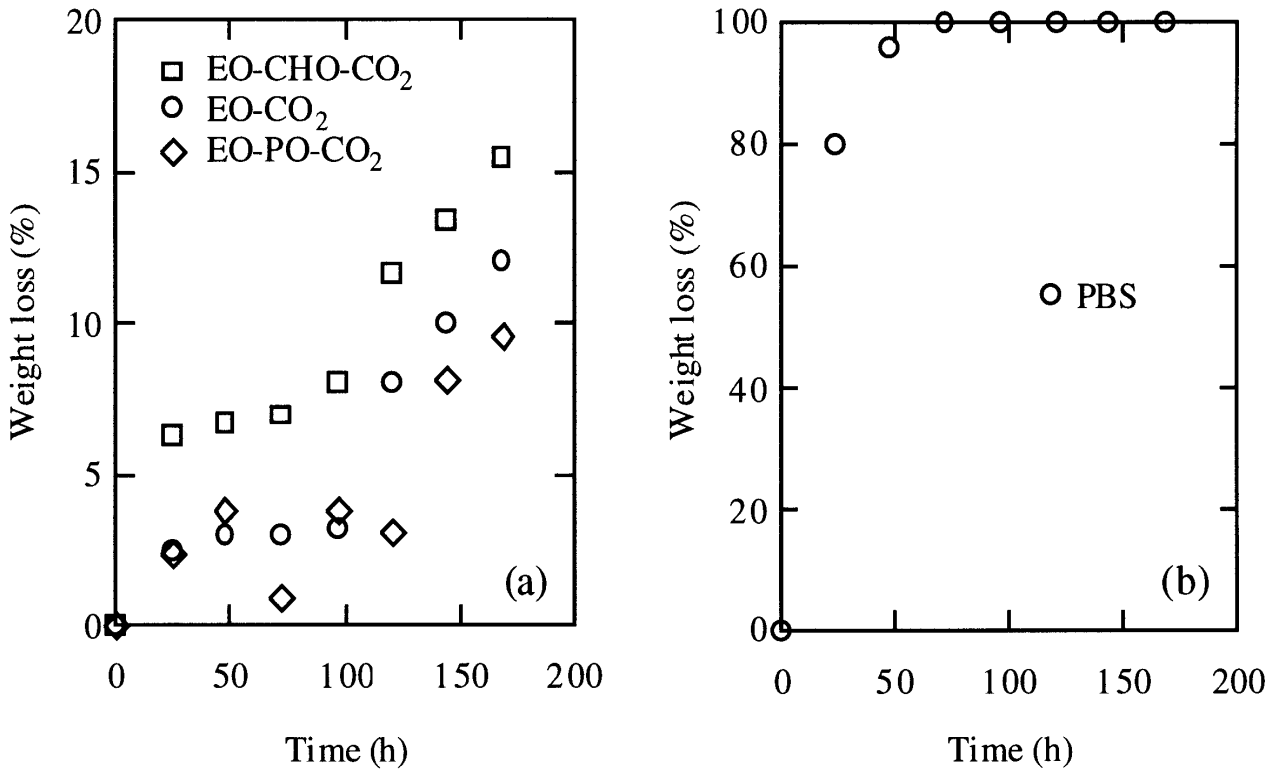
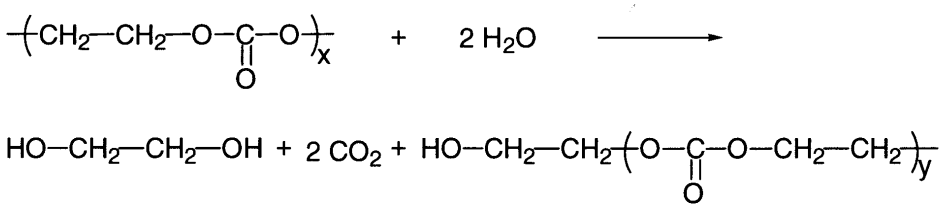


Fig 1 Time courses of the degradation of (a) polycarbonates [a] and (b) PBS [b] with *R. delemar* lipase at 37 ˚C

[a] Polycarbonate/*R. delemar*: 1 mg/10000U. [b] PBS/*R. delemar*: 1 mg/100U.

All these polymers were subjected to enzymatic degradation in the form of thin films, using two enzymes, *R. delemar* and *R. arrhizus* lipases, as catalysts. However, only three polycarbonates were hydrolyzed with the former enzyme which have oxyethylene units (Nos. 1, 4 and 5) and Tg values much lower than the degradation temperature of 37°C (Table 1). These results may suggest that the methyl group in PO and the cyclohexane ring in CHO exert steric hindrance to the enzymatic catalysis.

Figure 1a shows the time courses of the degradation of the three copolymers in an acetate buffer solution at 37°C with use of *R. delemar* lipase as catalyst. Their degradability as expressed by the weight loss was found to decrease in the following order: EO-CHO-CO_2 > EO-CO_2 > EO-PO-CO_2. All the copolymers lost their weight by 10-15% after 168 h degradation. As expected, ethylene glycol formed could be detected in the buffer solution and quantified by GLC after its conversion into the corresponding diacetate. On the other hand, when *R. arrhizus* lipase was used as catalyst, the degradation as determined by the weight loss method did not give any definite result. During the 168 h degradation the weight loss varied irregularly up to ca. 5%. Besides, no ethylene glycol could be detected in the reaction mixture after 168 h degradation. In sharp contrast to these results, films of PBS, a polyester, were completely degraded even after 72 h under similar conditions but with use of a much smaller amount of the enzyme (Fig. 1b). In the blank experiments without using any enzymes, no degradation of the copolymers and the polyester was observed in the buffer solutions.



Scheme 1 Hydrolysis of polycarbonates

Table 2 Relation between the weight losses of polycarbonate films and the yields of ethylene glycol [a]

No.	Polymer	Ethylene glycol (%) [b]	Weight loss (%)	
			Found	Calcd. [c]
1	EO-CO_2	7.0	12.1	9.1
2	EO-CHO-CO_2	32.0	15.4	14.1

[a] Hydrolysis with *R. delemar* at 37 °C for 168 h. [b] Ethylene glycol obtained by hydrolysis. [c] Weight loss calculated from the yield of ethylene glycol.

Table 2 summarizes the percent weight loss values and the yields of ethylene glycol after 168 h degradation with *R. delemar* lipase. Noteworthily, the degrees of degradation of polycarbonates are much smaller than those observed in our previous experiments *in vivo*, i. e., experiments by burying the copolymer pellet samples in the peritoneal cavity of rats (3), suggesting that the latter degradation is presumably due to the cumulative action of many kinds of enzymes. Further, comparison of the actual weight loss values with those calculated from the yields of ethylene glycol on the assumption that the hydrolysis takes place at inner ester groups (see Scheme 1) revealed that they are reasonably close to each other (see the last two columns). It should also be noted that the yield of ethylene glycol in the hydrolysis of ternary copolymer EO-CHO-CO_2 is much higher than that in the hydrolysis of binary copolymer EO-CO_2, while the weight loss values in the decomposition of the two polycarbonates are close to each other. These data indicate that the hydrolysis of the EO-CO_2 unit in the ternary copolymer proceeds about two times faster than that in the binary copolymer, even after allowance has been made for the lower content of the EO-CO_2 unit in the former. The reason for this acceleration remains to be established.

In summary, the present investigation has disclosed for the first time that aliphatic polycarbonates obtained from epoxides and CO_2 can certainly be degraded with *R. delemar* lipase at 37°C in an acetate buffer solution but much more reluctantly than poly(butylene succinate). Since aliphatic polycarbonates are environmentally benign in that they do not afford endocrine disruptors on degradation, their enzymatic biodegradability renders the aliphatic polycarbonates important polymeric materials not only from the viewpoint of

recycling CO_2 but also from the viewpoint of biodegradable plastics. Further work on the enzyme-catalyzed degradation of polycarbonates is in progress.

Acknowledgments

This work was supported by CREST (Core Research for Evolutional Science and Technology) of Japan Science and Technology Corporation (JST), and Bio-Nano Electronics Research Center, Toyo University. We thank Showa Highpolymer Co., Ltd., Tokyo, for a kind gift of PBS (Bionolle®).

References

1. a) Inoue S, Koinuma H, Tsuruta T (1969) Makromol Chem 130:210; b) Inoue S, Koinuma H, Yokoo Y, Tsuruta T (1971) Makromol Chem 143:97
2. Takanashi M, Nomura Y, Yoshida Y, Inoue S (1982) Makromol Chem 183:2085
3. Kawaguchi T, Nakano M, Juni K, Inoue S, Yoshida Y (1983) Chem Pharm Bull 31:1400
4. Nishida H, Tokiwa Y (1994) Chem Lett 421
5. Tokiwa Y, Suzuki T, Takeda K (1988) Agric Biol Chem 52:1937
6. Mukai K, Doi Y, Sema Y, Tomita K (1993) Kobunshi Ronbunshu 50:715