Hydrolysis of Polyesters by *Rhizopus delemar* Lipase

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We showed previously that several commercial lipases and hog liver esterase could hydrolyse synthetic polyesters, and that *Rhizopus delemar* lipase was capable of hydrolysing various kinds of polyesters.[1] We report here about some physical aspects effective to hydrolysis of polyesters by *Rh. delemar* lipase.

Poly(ethylene adipate) and polycaprolactone (PCL) were prepared by the method described previously.[2] Other polyesters, of which number average molecular weight ($\overline{M}n$) was measured by the terminal group determination method, were kindly supplied by Ube Industries, Ltd. Ultracentrifugally homogeneous preparation of *Rh. delemar* lipase was purchased from Seikagaku Kogyo Co., Ltd. Hydrolysis rate of polyesters was measured by the rate of their solubilisation, and the measurement process does not necessarily involve complete hydrolysis into the constituent parts. The solubilisation rate was determined at 30°C by measuring the total water-soluble organic carbon (TOC) concentration in the reaction mixture using a Toshiba-Beckman TOC analyser. X-Ray pattern was measured by using a Japan Electron Optics X-Ray Diffraction meter (Model JSDX–100S). Melting points of the polyesters (Tm) were measured by using a Yanaco Micro Melting Point apparatus (Model MP–S3).

The effect of the particle size of polyester powders on the rate of their enzymatic hydrolysis was examined. In the case of poly(ethylene adipate), small particles were hydrolysed better than large ones as shown in Fig. 1. So it was assumed that the enzymatic hydrolysis depends on the amounts of surface area of polyester powders. However, polyester powders prepared from a solution in chloroform by a precipitation method such as PCL, PCL-diol* and poly(hexamethylene adipate), were less susceptible to the enzymatic hydrolysis than their powders prepared by grinding at 30°C, in spite of the fact that the former particle size was much smaller than the latter one. Both powders gave the same X-ray diffraction diagrams which showed a high crystalline structure. When surfactant Plysurf** A 210G (Daiichi Kogyo Seiyaku Co., Ltd.) was added
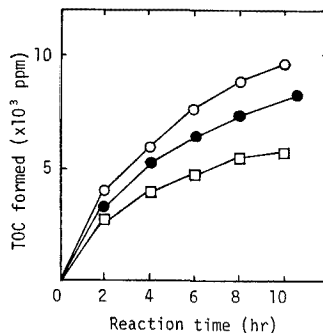
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* $H[O(CH_2)_5CO]_mO(CH_2)_6O[OC(CH_2)_5O]_nH$

** $RO-(CH_2CH_2O)_n-P\langle{}^{OA}_{OH}$ R, alkyl group;
             $\underset{O}{\overset{\|}{}}$
    A, –H or –(OCH₂CH₂)ₙ–OR

A, $-H$ or $-(OCH_2CH_2)_n-OR$

Fig. 1. Effect of the Particle Size of Poly(ethylene adipate) Powders on the Enzymatic Hydrolysis.

Each reaction mixture contained 400 μmoles of phosphate buffer (pH 6.0), 300 mg of poly(ethylene adipate) and 0.3 mg of *Rh. delemar* lipase in total volume 10 ml. In the substrate and enzyme controls, enzyme or substrate was omitted from the reaction mixture. Reaction mixtures were incubated on a rotary shaker at 180 rpm at 30°C. After incubation, the TOC concentration was measured. Particle size: ○—○, 0 ~ 0.25 mm; ●—●, 0 ~ 1.00 mm; □—□, 0.25 ~ 1.00 mm.

to the reaction mixture, the former was hydrolysed better than the latter. It was possible that other factors than the particle size and the crystallinity such as wettability might be involved in these cases.

Using three kinds of polyesters, PCL-diol, poly(1,6-hexamethylene adipate), and a copolyester made from 1,6-hexamethylenediol and a 70 : 30 molar ratio mixture of ε-caprolactone and adipic acid, the effect of molecular weight on the rate of their enzymatic hydrolysis was examined. $\overline{M}n$ did not affect the rate of hydrolysis by *Rh. delemar* lipase when $\overline{M}n$ was more than about 4000 (Fig. 2). This would indicate this lipase randomly splits ester bonds in polymer chains. In contrast, when $\overline{M}n$ was less than about 4000, the rate of the enzymatic hydrolysis was faster with the smaller $\overline{M}n$ of polyesters. This corresponded to the fact that the Tm was lower with the smaller $\overline{M}n$ of polyesters. Polyesters with low Tm would have less crystalline parts than those with high Tm.

The degree of crystallinity of polyester cannot be controlled by quenching. In order to study the effect of amorphous-crystal ratio on susceptibility of the enzymatic hydrolysis, copolymers of various molecular weights made from 1,6-hexamethylenediol and a 70 : 30 molar ratio mixture of ε-caprolactone and adipic acid were used. These copolymers were crystalline, but showed lower (Tm)s than the homopolymers. This would show that those copolymers have less order and more amorphous regions than the homopolymers do. The hydrolysis rates of these copolyesters with respect to only their chemical structure would be expected to
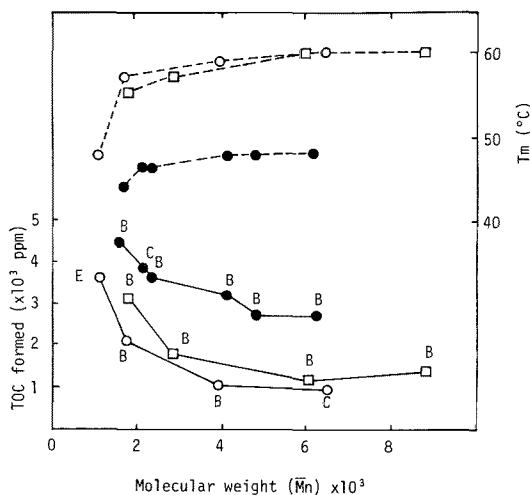
FIG. 2. Effect of Molecular Weight of Polyester on the Enzymatic Hydrolysis.

All polyesters were prepared into powder at 30°C by grinding. The particle size of each polyester powder was ranked A, B, C, D or E, corresponding respectively to roughly less than 0.25 mm, less than 0.50mm, less than 1.0 mm, 0.25~1.5 mm, 0.25~3 mm. The compositions of reaction mixture were the same as Fig. 1 except for addition of 300 mg of each polyester instead of poly(ethylene adipate). In the substrate and enzyme controls, enzyme or substrate was omitted from the reaction mixtures. The reaction mixtures were incubated for 16 hr in the same way as Fig. 1. Solid lines and broken lines indicated the rates of the enzymatic hydrolysis and melting points of polyesters (Tm) respectively. Symbol: —○—, poly(hexamethylene adipate); —□—, PCL-diol; —●—, copolyester made from hexamethylenediol and a 70:30 molar ratio mixture of ε-caprolactone and adipic acid.

be between, or near, the hydrolysis rates of the two homopolymers. But if amorphous parts of polyester were more easily hydrolysed than crystalline regions, the hydrolysis rates of the copolymers should be faster than those of the homopolymers. In practice, these copolymers were hydrolysed better than the homopolymers as shown in Fig. 2.
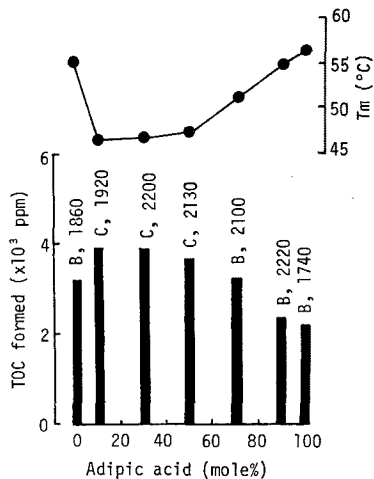
The enzymatic hydrolysis of other copolymers, with

FIG. 3. Effect of ε-Caprolactone and Adipic Acid Molar Ratio for Copolymers Made from 1,6-Hexamethylenediol and a Mixture of ε-Caprolactone and Adipic Acid on the Enzymatic Hydrolysis.

Reaction conditions were the same as Fig. 2. Four orders numbers in this figure showed $\bar{M}n$ of each polyester.

various molar ratios of ε-caprolactone and adipic acid, was also examined to see whether there was an optimum ratio of two components or not. $\bar{M}n$ of those copolymers was selected from 1740 to 2220, to diminish the effect of molecular weight. Optimum molar ratio of ε-caprolactone and adipic acid was about from 90:10 to 70:30 (Fig. 3). The Tm at the optimum molar ratio was the lowest of all. So it seemed that the existence of optimum molar ratio came from the lowest Tm, which would show the most amorphous material, rather than the optimum chemical structure.

From these results, it was concluded that the degree of crystallinity of polyesters would affect the rate of enzymatic hydrolysis.

REFERENCES

1) Y. Tokiwa and T. Suzuki, *Nature*, **270**, 76 (1977).
2) Y. Tokiwa, T. Ando and T. Suzuki, *J. Ferment. Technol.*, **54**, 603 (1976).