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Enzyme and Nonenzyme Hydrolyses of End Ester Group in Polyethylene Glycol Esters

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ABSTRACT

The enzyme and nonenzyme hydrolyses of the end ester group in polyethylene glycol (PEG) esters were studied. The end monoester in PEG ester is known to be easily hydrolyzed by alkaline or acidic solution. 2-Methyl-ω-benzoyloxypoly(oxy-1,2-ethanediyl)(Bz-PEG) and 2-methyl-ω-4-nitrobenzoyloxypoly(oxy-1,2-ethanediyl) were found to be hydrolyzed by lipase and esterase in Tris buffer solution (pH 7.7) but 2-methyl-ω-acetoxypoly(oxy-1,2-ethanediyl) was not. The parameters of enzyme reaction in the hydrolysis of ester bonds in Bz-PEG by esterase were estimated to be as follows: $V_{max} = 6.09 \times 10^{-7}$ mol/dm^3·s, $K_m = 7.63 \times 10^{-4}$ mol/dm^3.

INTRODUCTION

In recent years [1], polymeric drugs have been studied from the standpoint of the durability of their pharmacological activity; that is, the controlled release of medicine.

We synthesized an acryloyl-type polymer containing 5-fluorouracil (5-FU) residue through D-glucofuranose [2]. As a model experi-

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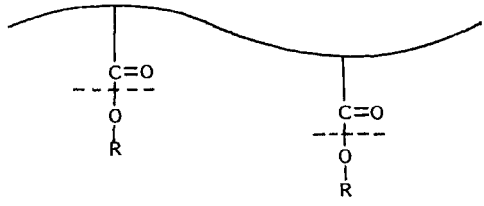
ment of the release of 5-FU residues from such a water-soluble acryloyl-type polymer, in the preceding paper [3] we dealt with the enzyme and nonenzyme hydrolyses of the pendant ester groups in a copolymer of p-nitrophenyl methacrylate and acrylamide as shown by Eq. (1).

In order to carry the medicine to the target region, the polymer support should be water-soluble. Polyethylene glycol (PEG) is biocompatible and water-soluble.

In the present paper the hydrolysis of the end ester group in PEG ester was used as a model reaction for the release of medicine from the end of the main chain as shown by Eq. (2).

(1)



(2)

EXPERIMENTAL

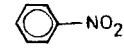
Materials

The PEG esters used are listed in Table 1.

2-Methyl-ω-acethyloxypoly(oxy-1,2-ethanediyl) (Ac-PEG) was prepared by reaction of polyethyleneglycol monomethyl ether (PEG-Me) with acetic anhydride, using pyridine as a scavenger of acetic acid, by modifying the Törmälä method [4] (bp 139.8-141.5°C/3.5 mm). 2-Methyl-ω-benzoyloxypoly(oxy-1,2-ethanediyl) (Bz-PEG) and 2-methyl-ω-4-nitrobenzoyloxypoly(oxy-1,2-ethanediyl) (p-NBz-PEG) were synthesized by reactions of PEG-Me with benzoyl chloride and 4-nitrobenzoyl chloride, respectively, using pyridine as a scavenger of hydrochloride, by the Inoue method [5]. 2-Methoxyethyl benzoate (Bz-EG) was prepared by reaction of ethylene glycol monomethyl ether (EG-Me) [6] (bp 94.8-95.5°C/4 mm, bp 110.5-110°C/7.5 mm).

Esterase and lipase, used as enzymes for the hydrolysis of ester bonds, were Sigma Chemical Co. type I from porcine liver and Sigma Chemical Co. type II from bovine pancreas, and used without purification.

TABLE 1. PEG Esters Used

PEG ester	n[a]	R
Ac-PEG	4	CH_3
Bz-PEG	4	
p-NBz-PEG	4	
Bz-EG	4	

[a] $CH_3(OCH_2CH_2)_nOC(=O)R$.

The 0.02, 0.025, and 0.05 M-tris buffer solutions (pH 7.7) were made up with special grade reagent and distilled water.

Kinetic Measurements

The enzyme hydrolysis of the ester group in the substrate was carried out in a Tris buffer solution of pH 7.7 at 37°C with shaking. The pH measurement was made on a Hitachi Horiba Model M-5 pH meter. The acidic and alkaline hydrolyses of the ester group in the substrate were carried out at 37°C in 0.01 N HCl and 0.01 N NaOH aqueous solutions respectively, with shaking. The extent of hydrolysis of PEG esters was determined by the reversed phase HLC method (column: TSK-GEL LS-400, eluting solution: methanol) or by means of titration of the acid produced.

RESULTS AND DISCUSSION

Nonenzyme Hydrolysis of the Ester Group in the Substrate

Assuming hydrolysis in the stomach (pH 2), the acid-catalyzed hydrolysis of the ester group in Ac-PEG was done at 37°C in 0.01 N HCl aqueous solution. The results in Fig. 1 show that Ac-PEG was hydrolyzed by an acid catalyst to give acetic acid. This result is different from that of the acid hydrolysis of the pendant ester groups in an acryloyl-type polymer; the latter polymer was only slightly hydrolyzed under the same condition [3].

On the other hand, the end ester group in Ac-PEG was found to be easily hydrolyzed in the alkaline solution as was the pendant ester groups in acryloyl-type polymer [3].

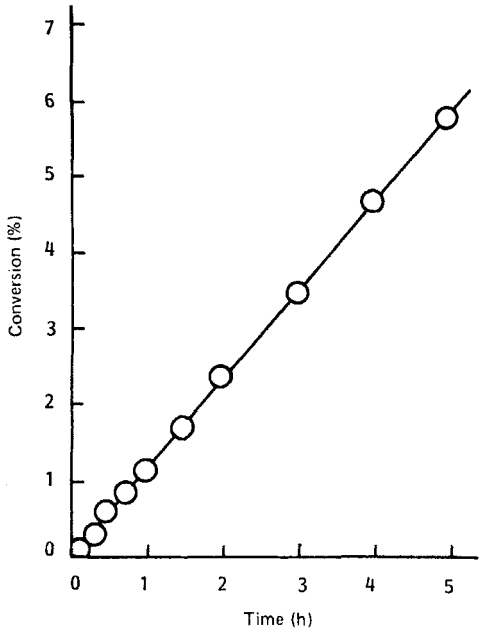


FIG. 1. HCl-catalyzed hydrolysis of the end ester group. Ac-PEG = 0.1 mol/dm^3, HCl = 0.01 mol/dm^3, in water, 37°C, with shaking.

Enzyme Hydrolysis of the End Ester Group in PEG Esters

The enzyme hydrolyses of end ester groups in PEG esters were investigated in 0.025 M Tris buffer solution (pH 7.7), using lipase as an enzyme. The results obtained are shown in Fig. 2. It is clear that Bz-PEG was easily hydrolyzed and p-NBz-PEG was slightly hydrolyzed, but Ac-PEG was not hydrolyzed at all.

The enzyme hydrolyses of end ester groups in PEG esters were studied in 0.05 M Tris buffer solution (pH 7.7), using esterase as an enzyme. The results obtained are shown in Fig. 3. Bz-PEG was hydrolyzed but Ac-PEG was not.

Such enzyme selectivity can be explained by the report [7] that these enzymes hydrolyze the hydrophobic substrate.

Comparison of Hydrolysis of Bz-PEG with Bz-EG

In order to understand the effect of the chain length of PEG, the esterase-catalyzed hydrolysis of monomeric Bz-EG was carried out

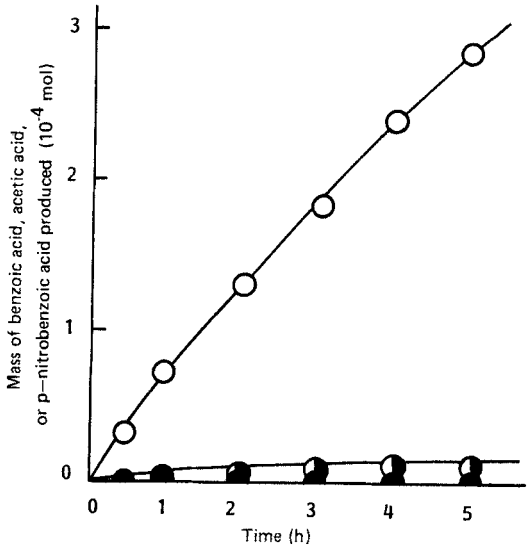


FIG. 2. Lipase-catalyzed hydrolyses of the end ester groups in PEG esters. Lipase = 100 units, 0.025 M Tris buffer solution (pH 7.7) = 10 cm^3, 37°C, with shaking. (○) Bz-$\overline{P}$EG = 1.18×10^{-3} mol, (◑) p-NBz-PEG = 1.09×10^{-3} mol, (●) Ac-PEG = 1.23×10^{-3} mol.

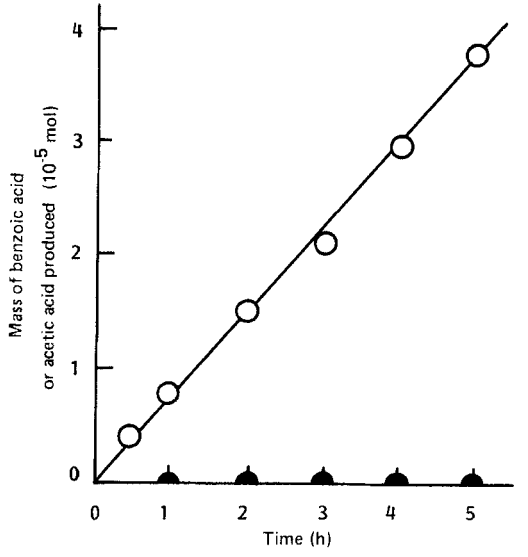


FIG. 3. Esterase-catalyzed hydrolyses of the end ester groups of Bz-PEG and Ac-PEG. Esterase = 1.74 units, 0.05 M Tris buffer solution (pH 7.7) = 10 cm^3, 37°C, with shaking. (○) Bz-$\overline{P}$EG = 7.75×10^{-4} mol, (●) Ac-PEG = 7.75×10^{-4} mol.

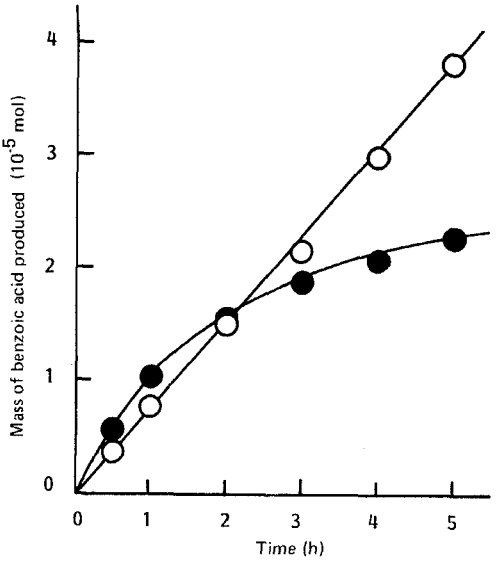


FIG. 4. Esterase-catalyzed hydrolyses of the end ester groups of Bz-PEG and Bz-EG. Esterase = 1.74 units, 0.05 M Tris buffer solution (pH 7.7) = 10 cm^3, 37°C, with shaking. (○) Bz-PEG = 7.75×10^{-4} mol, (●) Bz-EG = 7.75×10^{-4} mol.

under the same conditions. From Fig. 4 it is seen that the end ester of Bz-EG tends to be hydrolyzed more easily than that of Bz-PEG in the initial stage but is depressed as the reaction progresses. Such a depression of hydrolysis is considered to be attributable to the different kinds of alcohol produced. It is presumed that EG-Me, which is produced by the hydrolysis of Bz-EG, inhibited hydrolysis, but PEG-Me, which is produced by the hydrolysis of Bz-PEG, does not.

Additive Effects of EG-Me and PEG-Me on the Esterase-Catalyzed Hydrolysis of the End Ester Group of Bz-PEG

In order to confirm the inhibition of EG-Me produced by the enzyme-catalyzed hydrolysis of ester bond, the esterase-hydrolyses of Bz-PEG were carried out in the presence or absence of EG-Me and PEG-Me. The results obtained are shown in Fig. 5. As expected, EG-Me inhibited the esterase-catalyzed hydrolysis of the end ester group of Bz-PEG but PEG-Me did not. Thus, it is concluded that the selection of oligomeric PEG as a polymeric supporter is a good choice from the viewpoint of enzyme hydrolysis.

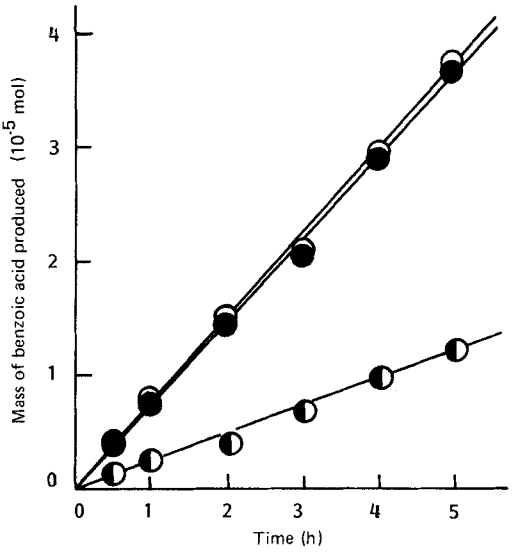


FIG. 5. Additive effects of EG-Me and PEG-Me on the esterase-catalyzed hydrolysis of the end ester group Bz-PEG. Bz-PEG = 7.75×10^{-4} mol, esterase = 1.74 units, 0.05 M Tris buffer solution (pH 7.7) = 10 cm^3, 37°C, with shaking. Additives: (○) None, (●) PEG-Me = 1.55×10^{-5} mol, (◐) EG-Me = 1.55×10^{-5} mol.

Adaptation of the Michaelis-Menten Mechanism

By keeping esterase in the feed constant at 174 units/dm^3 and varying the concentration of Bz-PEG as a substrate, the hydrolyses of the end ester groups of Bz-PEG were carried out at 37°C in 0.02 M Tris buffer solution (pH 7.7). The results obtained are shown in Fig. 6. Because of the good linear relationships at the initial reaction, we could obtain the velocities, V_0, from the slopes of these straight lines. Figure 7 shows the Lineweaver-Burk plots for the esterase-catalyzed hydrolyses of Bz-PEG. The results in Fig. 7 suggest that this hydrolysis proceeds through the Michaelis-Menten type of mechanism [8].

From the intercept of the ordinate at zero and from the slope of the straight line, the maximal velocity, V_{max}, and the Michaelis constant, K_m, were estimated to be 6.09×10^{-7} mol/$dm^3 \cdot s$ and 7.63×10^{-4} mol/dm^3, respectively. Such a value for V_{max} means that it was the proper hydrolysis velocity for the gradual release of medicine.

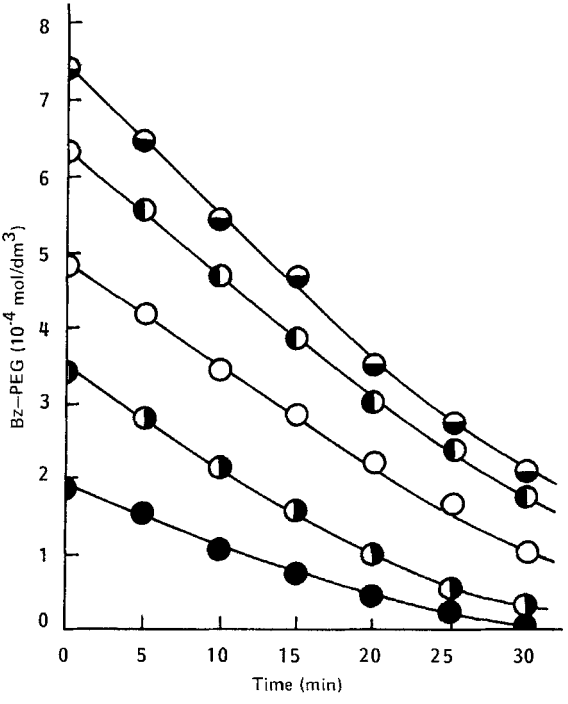


FIG. 6. Plots of [Bz-PEG] vs time for the esterase-catalyzed hydrolysis of the end ester group of Bz-PEG. Esterase = 174 units/dm^3 in 0.02 M Tris buffer solution (pH 7.7), 37°C with shaking. Bz-PEG = (◒) 7.42×10^{-4} mol/dm^3, (◐) 6.32×10^{-4} mol/dm^3, (○) 4.83×10^{-4} mol/dm^3, (◑) 3.43×10^{-4} mol/dm^3, (●) 1.86×10^{-4} mol/dm^3.

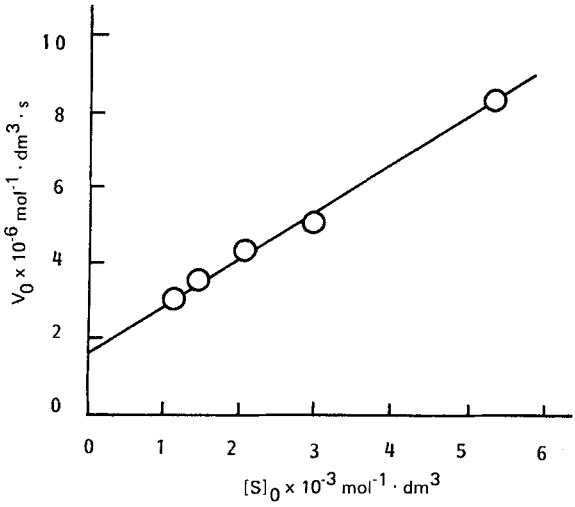


FIG. 7. Lineweaver-Burk plot for the esterase-catalyzed hydrolysis of the end ester group of Bz-PEG. Esterase = 174 units/dm^3 in 0.02 M Tris buffer solution (pH 7.7), 37°C, with shaking.

Thus, it is suggested that using PEG to bind a medicine to the end of a main chain by an ester bond is suitable for water-soluble polymeric drugs from the viewpoint of enzyme hydrolysis.

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