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POLYMERIZATION OF 10-HYDROXYDECANOIC ACID IN BENZENE WITH POLYETHYLENE GLYCOL-MODIFIED LIPASE

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SUMMARY

A hydrophobic substrate, 10-hydroxydecanoic acid having two functional groups (-OH and -COOH) in the molecule, was polymerized by ester bond formation with the polyethylene glycol-modified lipase in a transparent benzene solution. The polymer of 10-hydroxydecanoic acid was linearly elongated under a quite mild condition.

INTRODUCTION

We have shown previously that enzymes modified with polyethylene glycol dissolve easily in organic solvents and exert enzymic reactions (1-6). Since the water content in the reaction system can be reduced to minimum, various reactions have been very efficiently carried out, which are not generally observed in the presence of water; the modified lipase catalyzes ester synthesis, ester exchange and acid amide bond formation in benzene solution (2,4,5). The object of this paper is to extend our previous study on ester synthesis to the polymerization reaction with the modified lipase in a transparent benzene solution. With a substrate having two functional groups (-OH and -COOH) in the molecule, it is expected that the substrate will be polymerized by ester bond formation with the modified lipase.

MATERIALS AND METHODS

Crystallized lipoprotein lipase (EC 3.1.1.3) from Pseudomonas fluorescens was kindly provided by Amano Pharmaceutical Ltd., Nagoya, Japan. Monomethoxypolyethylene glycol with an average molecular weight of 5000 was obtained from Polyscience Inc., Warrington, Pa., USA. Other reagents used were of analytical grade.

The modifier, 2,4,-bis(O-methoxypolyethylene glycol)-6-chloro-s-triazine (activated PEG_2), was synthesized from monomethoxypolyethylene glycol and cyanuric chloride according to the procedure described previously (7). Amino groups of lipase were modified with activated PEG_2 as follows; activated PEG_2 (0.8 g) was mixed with lipase (20 mg) dissolved in 3.2 ml of 0.4 M borate buffer (pH 10.0). The reaction mixture was kept at 37°C for 1 hr under stirring, and the reaction was stopped by the addition of 100 ml of cold 0.1 M borate buffer (pH 8.0). The unreacted polyethylene glycol was removed by ultrafiltration with Diaflo PM-30 membrane (Amicon). The degree of modification of the lipase was 55%, which was estimated by measuring free amino groups with trinitrobenzene sulfonate (8). The modified enzyme was dialyzed against cold water and lyophilized. Protein concentration was determined by biuret method.

To 9.0 ml of benzene containing 25 mM 10-hydroxydecanoic acid was added 1.0 ml of benzene containing the modified lipase (1.0 mg protein/ml). The transparent mixture (10 ml) was incubated at 25°C and an aliquot (25 µl) was removed from the reaction mixture at a given time. The sample was subjected to gel permeation chromatography (GPC) in tetrahydrofuran at room temperature. The analysis was done with a Shimadzu LC-3A high performance liquid chromatograph using a Shodex GPC KF-803 column equipped with a reflactive index detector Shodex RI, Model SE-11. The flow rate was 1.0 ml/min.

RESULTS AND DISCUSSION

Figure 1 shows the GPC patterns of the reaction mixture at 0, 3 and 44 hr of the reaction time. At 3 hr the peak 1 (substrate) decreased and new peaks 2, 3, 4 and 6 appeared. At 44 hr the peak 1 completely disappeared and peak 6 became predominant. Without the modified lipase the substrate did not change at all. Although the retention time for the compound in peak 6 is longer than that in the peak 1 (substrate) on the GPC analysis, we believe that this is a high molecular weight polymer of the substrate from the following two experiments. It was hydrolyzed into the monomer and oligomers in an alkaline solution. The compound in the peak 6 did not pass through a dialysis membrane, while the substrate permeated freely. These results lead to the conclusion that polymerization of 10-hydroxydecanoic acid was accomplished with the modified lipase.

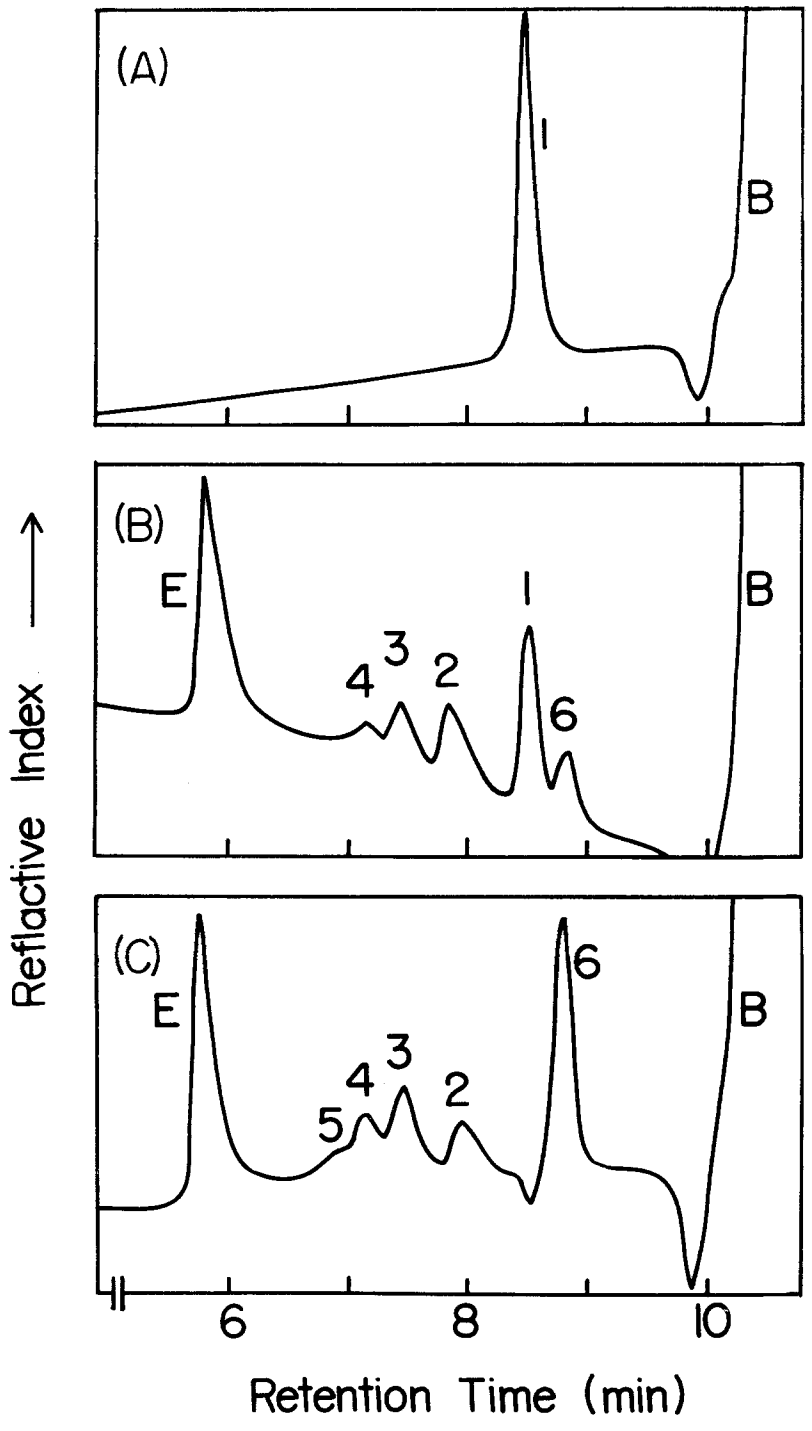

Fig. 1 Polymerization of 10-hydroxydecanoic acid with polyethylene glycol-modified lipase in benzene. GPC patterns of the reaction mixture at 0 (A), 3 (B) and 44 hr (C). Peaks B and E are benzene and the modified lipase, respectively.

This kind of abnormal behavior of polyesters on GPC columns has been encountered. For example, we have estimated the apparent molecular weight of polyethylene telephtalate to be 400-500 by this GPC column, which is supposed to have an average molecular weight of 19,700. It is possible that the anomaly of the compound in peak 6 is based on molecular structure of the polyester. If the molecule changes its gross structure from linear to globular, for instance, along with polymerization, their elution profile on GPC column would be quite complex. Moreover, as the substrate has two reactive groups at both ends, cyclic products can be expected. Every products, however, retained a carboxylic group reactive with Adam reagent (9), suggesting that the polymer is only linearly elongated. Detailed characterization of products will be revealed.

We have shown in this article that enzymic polyester synthesis is possible in benzene under the quite mild condition.

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