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Esterification and Transesterification With Immobilized Lipase in Organic Solvent†

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ABSTRACT

In this paper, we report the reactions of esterification and transesterification catalyzed by the following lipase adducts in organic solvents: (*1*) glass-adsorbed; (2) acetone precipitated on porous glass, kieselghur-adsorbed; (3) Al_2O_3-adsorbed; and (*4*) agar bead-adsorbed. The optimal water content varied for different forms of the enzymes. Under the most favorable conditions, kieselguhr-adsorbed and agar bead-adsorbed lipases, which have higher catalytic activities in organic solvents, are the best of all forms of lipases.

Index Entries: Lipase; immobilization; esterification; transesterification.

INTRODUCTION

Research on enzyme catalytic reactions in organic phase has been an active area in recent years. We have made systematic studies on esterification and transesterification of powdered lipase in organic solvents. We studied reactions catalyzed by immobilized lipases in organic solvents.

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MATERIALS AND METHODS

Materials

1. Porcine pancreatic lipase was purchased from Sigma Chemical Company (L 3126).
2. Porous glass, aluminum oxide, and Kieselguhr were made in China.
3. Other chemicals were of analytical grade made in China.

Methods

1. Lipase activity in water phase was determined by the hydrolysis reaction of olive oil (*1*).
2. Lipase activities for esterification and transesterification were determined by the following steps. Enzyme and substrate were mixed in organic solvent. The reactions were carried out at 40°C for about 24 h under stirring. Activity is measured by high-pressure liquid chromatography (HPLC) according to the decrease of substrate peak area or increase of product peak area (Fig. 1, 2).
3. Determination of protein concentration was carried out by the MicroBiuret reaction using BSA as a standard (*2*).
4. Removal of water from organic phase was performed with $CaCl_2$ and 4 A molecular sieve (*3*).
5. Lipase immobilization:
   a. Adsorption of lipase with porous glass powder, kieselguhr, and aluminum oxide: 0.5 g lipase was dissolved in 5.0 mL of 0.02 mol/L phosphate buffer (pH 7.7); 2.0 g different carriers were added to the above solution respectively under stirring, then filtrated, and lyophilized.
   b. Precipitated lipase on porous glass powder with acetone: 0.5 g lipase was dissolved in (A) buffer and 2.0 g of porous glass was added to the above solution. Next, 5 mL of cold acetone was added slowly under agitation at 4°C. The solution was filtrated after 30 min, and the immobilized enzyme was washed three times in cold acetone, and lyophylized.
6. Adsorption of lipase with *p*-methylphenylamine agar bead:

   p-Methylphenylamine was introduced to agar beads by the tricyanogen chloride-activation method. Ten g wet p-methylphenylamine agar bead and 1.0 g of lipase powder were added to 20 mL of Na_2HPO_4/Citric acid buffer (pH 5.0, 0.2 mol/L), and the mixture was incubated at 15°C for 5 h, then filtrated, washed three times with phosphate buffer (pH 7.7), and lyophylized.

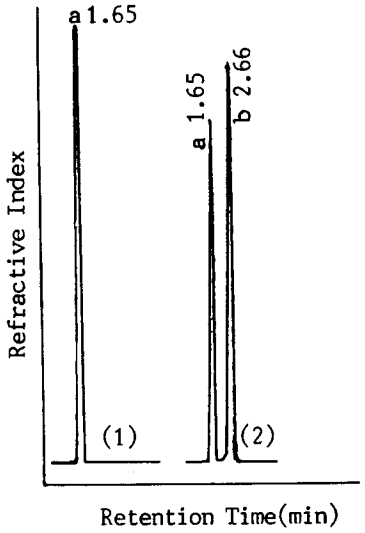

Fig. 1. Determination of esterification by HPLC. A mixture of lauric acid (0.8 mol/L, 0.45mL) and lauryl alcohol (1.2 mol/L, 0.45mL) was incubated at 40°C for 24 h with lipase (4.0 mg protein) (2) or without lipase (1). The analysis was performed using C_{18} column in 80% tetrahydrofuran, and the flow rate was 1.0 mL/min. Peak a: lauric acid and lauryl alcohol. Peak b: lauryl laurate.

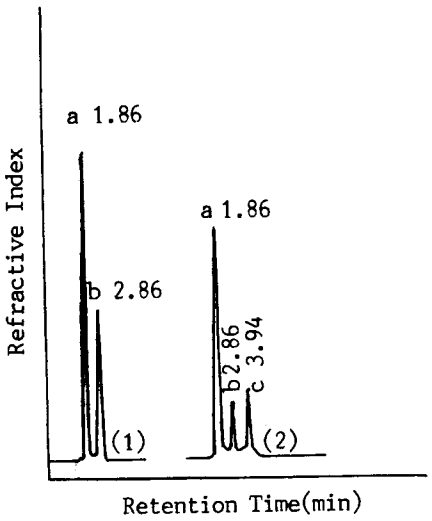

Fig. 2. Determination of transesterification reaction by HPLC. A mixture of butyl stearate ((0.8 mol/L, 0.45 mL) and lauryl alcohol (1.2 mol/L, 0.45 mL) was incubated at 40°C for 24 h with lipase (4.0 mg protein) (2) or without lipase (1). The analysis was performed using C_{18} column in 80% tetrahydrofuran, and the flow rate was 1.0 mL/min. Peak a: lauryl alcohol. Peak b: butyl stearate. Peak c: lauryl stearate.

RESULTS AND DISCUSSION

The Relationship Between Activity of Immobilized Lipase and Water Content

The activities of glass-adsorbed and acetone-precipitated lipases for transesterification of butyl stearate with laurinol were measured in benzene with different water amounts. The results were shown in Table 1.

Kieselguhr-, aluminum oxide-, agar bead-adsorbed enzymes catalyzed the same reactions as above. The activities with different water content were as Table 2. In this system, kieselguhr-, aluminum oxide-, and agar bead-adsorbed lipase had high activities in 1.0, 10.0, and 0–0.4% water, respectively.

The results show that water is a necessary factor for enzyme activity, and various forms of the immobilized enzyme had optimal water content, respectively. Water in the reaction system not only provided water for the carrier and solvent and made the saturation of carrier and solvent, but it also satisfied the essential water layer on the surface of the enzyme.

Comparison of Various Forms of the Immobilized Enzyme

Transesterification Reaction
(Butyl Octadycylate and Dodecanoic Alcohol)

The immobilized enzyme catalyzed transesterification reaction in benzene with optimum water content. The results are shown in Table 3.

Esterification Reaction
(Dodecanoic Acid and Dodecanoic Alcohol)

Various forms of lipase-catalyzed esterification in benzene with optimum water content. The results are shown in Table 4.

The results show that agar bead hydrophobic-adsorbed lipase had good catalytic function by comparison to various forms of lipase. It increased catalytic activity of lipase in organic phase. Its activity increased 46.5% compared to powdered lipase. The reason for the activity increase is that the hydrophobic substrate centered near the hydrophobic carrier due to the hydrophobic interaction made interaction between lipase and substrate increased; and also because the enzyme immobilized in hydrophobic carrier had better stabilization than that in hydrophilic carrier (*4–8*). After all, we explored a new and effective type of immobilized enzyme and first used it in the organic phase reaction. Hydrophobic carrier as a part of catalyst affected the activity and stability of the enzyme by controlling the microenvironment of the enzyme.

Table 1
Relationship Between the Activity of Glass-Adsorbed and Acetone-Precipitated Lipase and the Water Amount Added to the System*

Water content % (v/v)		0.0	0.2	0.4	0.6	0.8	1.0
Activity ($\times 10^2$)	Glass–acetone-precipitated lipase	3.0	4.4	6.3	7.5	6.0	3.9
μmol/min·mg Pr	Glass-adsorbed lipase	3.0	3.3	3.8	7.2	4.9	3.0

*The highest activities of both lipases were obtained in benzene with 0.6% water.

Table 2
Relationship Between the Activity of Immobilized Lipases and the Water Amount Added to the System

Water content % (v/v)		0.0	0.4	0.8	1.0	1.2	1.6	2.0	3.0	5.0	10.0	15.0
Activity ($\times 10^2$) μmol/min·mg Pr	Kieselguhr-adsorbed lipase	10.0			11.2			3.7	6.9	5.5	2.6	0.0
	Al_2O_3-adsorbed lipase	0.0	0.0	0.0		0.0	0.0	0.0		7.4	8.6	0.0
	agar bead-adsorbed lipase	16.7	16.7	16.0		15.9	12.1	10.8	9.8	9.5	8.0	

Table 3
Enzymatic Activities of Various Forms of Lipase in Transesterification

Forms of lipase	Water content % (v/v)	Activity ($\times 10^2$) μmol/min·mg Pr
Powdered lipase	0.0	9.2
Lyophilized, powdered lipase in opt. pH	0.0	11.4
Glass–acetone-precipitated lipase	0.6	8.7
Glass-adsorbed lipase	0.6	8.5
Kieselguhr-adsorbed lipase	1.0	11.2
Al_2O_3-adsorbed lipase	10.0	8.6
Agar bead-adsorbed lipase	0.0	16.7

Table 4
Enzymic Activity for Esterification Reaction with Various Forms of Lipase

Forms of lipase	Powdered lipase	Lyophilized, powdered lipase in opt. pH	Agar bead-adsorbed lipase
Activity ($\times 10^2$) μmol/min·mg Pr	2.28	2.30	2.48

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