0273-2289/92/32(1/3)—0007$02.00

Lipase Catalysis in Organic Solvents†

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Received August 1, 1991; Accepted September 12, 1991

ABSTRACT

The conditions for esterification and transesterification catalyzed by porcine pancreatic lipase in organic media were studied. It was found that the enzyme reaction was dependent on the following factors: the pH at which the enzyme powder was prepared from its solution, the polarity of organic media, the reaction temperature, the water content in reaction system, and the substrate structures. Effects of the above factors on enzyme activity were discussed.

Index Entries: Lipase; esterification; transesterification; biocatalysis; organic media.

INTRODUCTION

An increasing interest in the problems of biocatalysis in nonaqueous media has been evident in recent years because biocatalysts (enzymes) possess extremely high catalytic activity and unique substrate specificity (*1,2*). It is these merits that make the application of enzymes in fine organic synthesis extremely promising. However, wide application of biocatalysis in this biotechnological area is hindered by the idea that enzymes are believed to function effectively only in water-rich media. On the other hand, in classic organic synthesis, it is various organic solvents that are

†This work was presented at the China-Japan Symposium on Enzyme Engineering, held October 22–25, 1990, at Wu Xi, China.

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generally used as reaction media. Therefore, improvement of the enzymatic activity and stability in organic media is a prerequisite of extending enzyme application scope. In organic solvents, the enzymatic properties, catalytic reaction, and the factors that affect enzymatic activity and stability are all different from that in water phase. The presence of organic solvents makes it possible for enzymes to possess a new catalytic behavior, for example, lipase catalytically hydrolyzes oil or fat in water media; whereas the same enzyme catalyzes esterification and transesterification in organic media, obtaining chiral compounds such as macrocyclolactone etc., which is difficult to secure using chemical methods (*3*).

In this paper, the factors that affect esterification and transesterification catalyzed by lipase in organic solvents are studied. This kind of study is important not only as a basis of improving lipase activity in organic solvents for chemical production but also reveals enzyme action mechanism in hydrophobic conditions and provides a new method and information for the study of enzyme structure and function.

MATERIALS AND METHODS

Materials

Porcine pancreatic lipase was purchased from Sigma Chemical Company. Specific activity of the enzyme is 1170 units/g solid. Lauric acid, laurinol, and other chemicals were all of analytical grade.

Methods

1. Determination of Esterification Activity

Lauric acid (0.45 mL of 0.8 mol/L) and laurinol 0.45 mL of 1.2 mol/L were added to organic solvents; then lyophilized enzyme powder (4.0 mg protein) was added to the above mixture under stirring and incubated at 40°C for 24 h. The solution was centrifuged obtaining supernatant for assay. Esterification activity was calculated from the increase of area of product peak, which was determined by high-pressure liquid chromatography (HPLC). One unit of enzyme activity is defined as the amount of enzyme that liberates 1 μmol of product or that will decrease 1 μmol of substrate/min.

2. Determination of Transesterification Activity

Butyl stearate (0.45 mL of 0.8 mol/L) and laurinol 0.45 mL of 1.2 mol/L were added to organic solvents, then lyophilized enzyme powder (4.0 mg protein) was added to the above mixture under stir. Determination of transesterification activity was carried out according to the Method 1. One unit of enzyme was defined as the amount of enzyme releasing 1 μmol of product from the substrate/min.

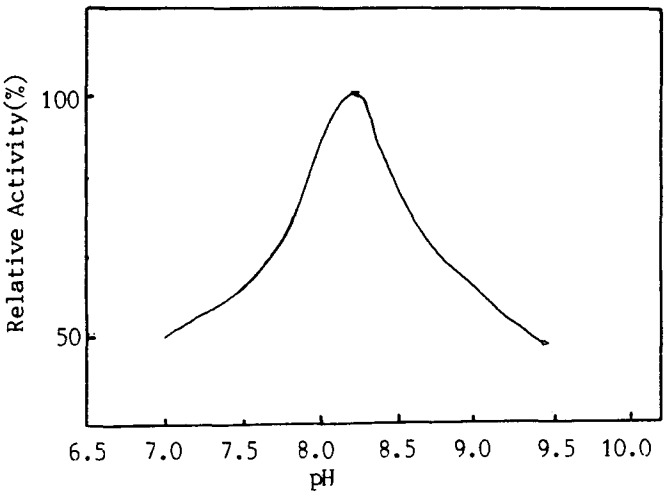

Fig. 1. The relationship between lipase activity and pH condition.

3. Preparation of Enzyme Powders from Enzyme Solutions with Different pH

Crude enzyme powders were dissolved in 0.02 mol/L phosphate buffers with different pH and centrifuged. The supernatants were lyophilized into enzyme powders.

RESULTS AND DISCUSSION

Effect of pH on Enzyme Activity

Transesterification reactions of butyl stearate with laurinol were carried out at 20°C in water-free benzene by using a series of enzyme powders prepared according to Method 3. The results (Fig. 1) show that enzyme activity in an organic solvent is dependent on the pH at which the enzyme powder was prepared from the aqueous solution. The highest transesterification activity in organic solvents corresponds to the pH that was optimal for enzymatic activity in water. This is explained by the fact that enzymes only need a thin layer of water on the protein surface to retain their catalytically active conformation in anhydrous media (*4*). The ionogenic groups of the enzyme molecule acquire a certain ionization state in the aqueous solution of a given pH; and that ionization state—the enzymatic activity corresponding to it—is retained in a solid state in organic solvents.

Effect of Water Content in the System on Enzyme Activity

The highest transesterification activity of enzyme powder in water-free benzene was obtained at 20°C; its activity was 8.0 mol/min·mg pro-

Table 1
Activity of Lipase in Various Organic Solvents

Organic solvents	Constant of polarity (lgP)	Activity ($\times 10^2$) μmol/min·mg Pr
Ethanol	−0.24	0.0
Acetone	−0.23	0.0
Butanol	+0.28	0.0
Ethylacetate	+0.68	0.0
Benzene	+2.00	10.6
Toluene	+2.50	13.5
Pentane	+3.00	11.4
Hexane	+3.50	10.5
Heptane	+4.00	6.5
Dodecane	+6.60	6.1

tein. When 0.2% (v/v) of water was added into the system, enzyme activity was decreased, and enzyme powder cohered. When 0.6% (v/v) of water was added, enzyme powder was completely gathered, and no activity was displayed. This phenomenon indicated that transesterification reaction needs a hydrophobic environment.

Effect of the Polarity of Solvents on Enzyme Activity

Esterification activities of butyl stearate with laurinol catalyzed by lipase in organic solvents with different polarity constants (lgP) are listed in Table 1. The enzyme worked well in organic solvents with polarity constants ranging from 2.0 to 3.5, but the enzyme did not work in water-miscible solvents, such as, alcohol and acetone.

In dodecane with lgP 6.6, the enzyme activity was not high, only 57.5% of that in benzene. This is in contradistinction to reference (*6*). The reason may be that the substrate is hydrophobic, and its hydrophobility is so high that the substrate does not easily diffuse into the surface of enzyme molecule. Organic solvents with lgP 2.0–3.5 do not strip the essential water from enzymes and do not interfere with the substrate diffusion, so the enzyme may function well.

Effect of Temperature on Lipase Activity and Its Thermostability

The transesterification activities of butyl stearate with laurinol catalyzed by lipase in water-free benzene are listed in Table 2. The results show that enzyme activity increased with temperature increase.

Table 2
Activity of Lipase at Different Temperatures

Temperature (°C)	20	25	37	50	60	70
Activity ($\times 10^2$) μ mol/min·mg pr.	8.0	8.3	9.0	9.8	10.6	11.8

Table 3
Relationship Between the Activity of Powdered Lipase and the Number of Reaction

No. of reaction		1	2	3	4	5	6	7
Relative activity (%)*	40°C	100	98	95	90	85	81	77
	70°C	100	98	91	87	81	75	60

*At 40°C, 100% activity represents 9.2×10^{-2} μmol/min·mg Pr; at 70°C, 100% activity represents 11.8×10^{-2} μmol/min·mg Pr.

The thermostability of the enzyme was determined by using continuous batch reaction method in water-free benzene. The result, shown in Table 3, indicate that enzyme thermostability was high: Enzyme activities remained 77 and 60%, respectively,when the reaction was carried out at 40 and 70°C, respectively; and continuously proceeded seven times with each batch for 96 h.

The reasons why lipase is so stable in organic solvents may be that the denaturation process of enzymes requires water and therefore should not occur in a water-free environment or that dehydration makes lipase much more rigid and eliminates its conformational flexibility (4,7). Therefore, enzymes in organic solvents keep their appropriate folded conformations even in the high temperatures.

Effect of Substrate Structure on Enzymatic Activity

Data on esterification reactions of laurinol to various fatty acids and of lauric acid to various fatty alcohols catalyzed by lipase in water-free benzene are listed in Tables 4 and 5, respectively. The results show that as the carbon chain length of fatty acids and fatty alcohols increases the rate of enzyme reaction increases, but the rate decreases when the chain length of fatty acids is more than 12 carbons. The primary alcohol is superior to second alcohol in reactivity. In addition, it is the fatty alcohols with more than six carbons that can be esterified by hexylic acid.

Data on transesterification reactions of methyloleate with various fatty alcohols catalyzed by lipase in water-free benzene are listed in Table 6. The rate of transesterification was highest when laurinol as substrate.

Table 4
Reactions of Laurinol to Various Acids

Fatty acid	Activity ($\times 10^2$) μmol/min·mg Pr)
Hexanoic acid (C_6)	2.94
Octanoic acid (C_8)	3.43
Dodecanoic acid (C_{12})	5.36
Palmitic acid (C_{16})	4.91
Linoleic acid (C_{18})	4.86
Arachic acid (C_{20})	4.76

Table 5
Reactions of Lauric Acid and Hexanoic Acid to Various Fatty Alcohols

	Activity ($\times 10^2$ μmol/min·mg Pr)	
Fatty alcohols	Lauric acid	Hexanoic acid
Butanol (C_4)	2.96	0.00
Pentanol ($_5$)	3.49	0.00
Hexanol (C_6)	3.65	0.00
Cyclohexanol (C_6)	1.88	0.00
Heptanol (C_7)	4.00	1.42
Nonanol (C_9)	4.90	1.94
Laurinol (C_{12})	5.36	1.46
Octadecyl alcohol (C_{18})	6.15	1.67

Table 6
Reaction of Methyloleate to Various Fatty Alcohols

Fatty alcohols	Hexanol	Nonanol	Laurinol	Octadecyl alcohol
Activity ($\times 10^2$ μmol/min·mg Pr)	2.21	2.63	3.02	2.79

The above results indicate that esterification and transesterification activities of lipase in water-free benzene are intimately associated with substrate structure (carbon chain length). This is because the chain length of substrate directly influenced the hydrophobic affinity and structure fit between substrates and enzyme molecules. Fatty glyceride is native hydrophobic substrate for lipase that has highly hydrophobic active site; therefore, the substrates with high hydrophobility (long carbon chain) easily attach to enzyme molecules. However, the spatial hindrances of substrate chains also affects the structure fit between enzyme and substrate.

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