Biochemical Engineering Journal 25 (2005) 39–45

Biochemical Engineering Journal

www.elsevier.com/locate/bej

The mechanism and kinetic model for glycerolysis by 1,3 position specific lipase from *Rhizopus arrhizus*

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Received 23 January 2005; accepted 4 March 2005

Abstract

Hydrolysis and glycerolysis of l,3-palimitin-2-olein (POP) was studied and mechanism of glycerolysis was discussed. The results showed that the glycerolysis is a complicated process including hydrolysis, esterification and isomerization of monoglycerides (MG) and diacylglycerols (DG). At first 5 h of glycerolysis, hydrolysis dominates the process and then esterification and isomerization will take place. A new mechanism and kinetic model based on hydrolysis, esterification and isomerization were established to correct all reactions in glycerolysis. The average relative error between experimental data and simulated results is 8.37%. The kinetic model can be used to predicate the results of glycerolysis at different molar ratios of POP to glycerol. The relative errors between the results predicted and experimental data are lower that 10%, which means that the kinetic model can be used to describe for glycerolysis of triglycerides.
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Keywords: Lipase; 1,3-Palmitin-2-olein; Glycerolysis; Mechanism; Kinetic model

1. Introduction

Glycerolysis of triacylglycerol can produce many useful products such as monoglycerides (MG) and diacylglycerols (DG), which have wide applications in food, cosmetics and pharmaceuticals [1]. The glycerolysis carried out by a lipase has got attention due to its mild reaction conditions and position specific products such as 2-monoglycerol by 1,3 specific lipase [2]. Yamane and his co-workers studied the production of MG by a glycerolysis with a lipase from *Pseudomons fluoresc* and their results showed that the conversion was strongly dependent on reaction temperature [3,4]. The 60–90% conversion could be obtained below critical temperature.

Millqvist et al. used 1,3 specific lipase from *Rhizopus* to produce 2-monoglyceride and high conversion (92%) was obtained [2]. Glycerolysis by different systems, such as two-phase system [5] and reverse micelles system [6], have also been reported.

Although a lot of applications of glycerolysis by lipase catalysis have been seen, until now a few were concerned with the mechanism and kinetic model on glycerolysis. Boswinkl et al. suggested acyl migration in monoglycerol synthesis to explain formation of 2-monoolein [7]. The result showed that the acyl migration rate was strongly dependent on the acyl chain length. Peng et al. suggested a mechanism including hydrolysis, esterification and interesterification for glycerolysis [8]. However, this model is rather complicated and there is no experimental data to support it. Heisler et al. studied the glycerolysis of tripalmitin by 1,3 lipase, and found the isomerization of 1,2-dipalmitin into 1,3-dipalmitin [9]. He presented a two-step mechanism for glycerolysis. The first step is hydrolysis of dipalmitin and then an isomerization of 2-monopalmitin into 1-monopalmitin happens. But the detailed mechanism and kinetics of glycerolysis have not been discussed.

Lykidis et al. [10] and Dandik et al. [11] studied the kinetics of hydrolysis of triacylglyerol by a lipase. All these models are a two-step mechanisms, triacylglycerols were firstly hydrolyzed to 1,2 diacylglycerol, then the diacylglycerol was subsequently converted into 2-monoacylglycerol. Kinetics of interesterification of triacylglycerol has also been reported and a Ping–Pong model was presented [12]. Until now, to the best of our knowledge, the mechanism and kinetics of

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1369-703X/$ – see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.bej.2005.03.009

Nomenclature

MG	monoglyceride(s)
DG	diglyceride(s)
TG	triglyceride(s)
FFA	fatty acid(s)
C	molar concentration (mol/cm^3)
POP	1,3-palmitin-2-olein
POO	l,2-olein-3-palmitin
OOO	triolein
POG	l-palmitin-2-olein
PGO	1-palmitin-3-olein
OOG	1,2-diolein
OGO	1,3-diolein
PGP	1,3-dipalmitin
PG	1-monopalmitin
OG	1-monoolein
GO	2-monoolein
PA	palmitic acid
OA	oleic acid

glycerolysis have not been reported. In this paper, the mechanism was studied and a new kinetic model for glycerolysis was established.

2. Materials and methods

2.1. Materials

Rhizopus arrhizus strain was stored in our laboratory. Chinese vegetable tallow (CVT) was purchased from Nanjing Grain and Oil Company (Nanjing, China). Glycerol and other chemicals without special mention were of analytical grade.

Acetonitrile and methylene chloride of HPLC grade were obtained from Caledon Laboratories Ltd. (Georgetown, Ont., Canada). Acetic acid was of analytical grade from Beijing Chemicals Factory (Beijing, China).

The following reference standards were purchased from Sigma Chemical (St. Louis, MO, USA): palmitic acid, oleic acid, 1-monopalmitoyl-rac-glycerol, 2-monopalmitoylglycerol, 1-monoolein, 2-monoolein, 1,2-diolein, 1,3-diolein, 1,2-dipalmitin, 1,3-dipalmitin, 1,3-dipalmitoyl-2-oleoyl-glycerol, 1,2-dioleyl-3-palmitoyl-glycerol and triolein. 1-Palmitin-2-olein and l-palmitin-3-olein were prepared by our laboratory with a purity of 98%.

2.2. Preparation of R. arrhizus lipase

The ingredients of culture medium for *R. arrhizus* were (w/w): soybean flour 4.0%, earthnut oil 1.0%, $MgSO_4$ 0.1%, K_2HPO_4 0.5% and $(NH_4)_2SO_4$ 0.2%. The culture was carried out at 26.5 °C in a shaker (130 rpm) for 70 h [13]. The broth was centrifuged to remove the cells and the lipase in the supernatant was precipitated by three volumes of cool acetone (−10 °C). The precipitate was washed by double volumes of cool acetone three times and dried at the room temperature. The activity of lipase powder was 6000 u/g.

2.3. Preparation of 1,3-palmitin-2-olein (POP)

The crude CVT contained 70% 1,3-palmitin-2-olein (POP) and other higher melting compositions, such as tripalmitin. It could not be directly used as glycerolysis substrate in solvent-free system for its higher melting point (60–65 °C). In this paper, we refined 1,3-palmitin-2-olein from crude CVT and used it as the substrate of glycerolysis.

Firstly, the crude CVT was refined by crystallization in acetone: 100 g crude CVT was dissolved in 600 ml acetone at 34 °C, then the mixture was cooled to 21 °C at the rate of 2 °C h^{-1} and was held at 21 °C for 10 h to ensure that high melting point fractions were thoroughly precipitated. The precipitate was removed by a vacuum filtration. The acetone in the filtrate was removed by using a rotary evaporator to obtain a colorless oil mixture. Then the colorless oil mixture was collected and was re-crystallized in 1:1 (v/v) ether:hexane. The mixture was dissolved in 7 volumes of 1:1 (v/v) ether:hexane at 14 °C, then cooled to 8 °C at the rate of 2 °C h^{-1} and held at this temperature for 8 h, the formed precipitate was removed by vacuum filtration. The solvent was removed by a rotary evaporator, and the residue was refined CVT that contained 96% POP.

The POP was treated with alumina to remove traces of MG, DG, fatty acids (FFA), oxidation products and water [14]. The melting point of the POP product was about 35 °C.

2.4. Glycerolysis reaction

POP (l0 g, 12 mmol) and an appropriate amount of glycerol (if not specially mentioned, containing 10 wt.% water) as well as free *R. arrhizus* lipase powder (250 u/g oil) were added into a 50 ml conical flask with a rubber cap. The mixture was incubated in a water bath with a magnetic stirring (800 rpm) at 37 °C.

2.5. Hydrolysis reaction

The hydrolysis experiments were similar to the glycerolysis, except that no glycerol was added: 0.23 g water and 10 g POP (12 mmol) as well as free *R. arrhizus* lipase powder (250 u/g oil) were added into a 50 ml conical flask with a rubber cap. The mixture was incubated in a water bath with a magnetic stirring (800 rpm) at 37 °C.

2.6. Enzyme activity assay

Activity of lipase was determined by an olive oil emulsion method [13]. One unit of activity is defined as the amount of

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