KINETIC MODEL FOR TRIGLYCERIDE HYDROLYSIS USING LIPASE: REVIEW

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Abstract

Triglyceride hydrolysis using lipase has been proposed as a novel method to produce raw materials in food and cosmetic industries such as diacylglycerol, monoacylglycerol, glycerol and fatty acid. In order to design a reactor for utilizing this reaction on industrial scale, constructing a kinetic model is important. Since the substrates are oil and water, the hydrolysis takes place at oil-water interface. Furthermore, the triglyceride has three ester bonds, so that the hydrolysis stepwise proceeds. Thus, the reaction mechanism is very complicated. The difference between the interfacial and bulk concentrations of the enzyme, substrates and products, and the interfacial enzymatic reaction mechanism should be considered in the model.

Keywords: Lipase, kinetic model, enzymatic reaction mechanism, hydrolysis, triglyceride

1. Introduction

Triglyceride, the main component of natural oil or fat, is stepwise converted into diacylglycerol, monoacylglycerol and glycerol by hydrolysis accompanied with the liberation of a fatty acid at each step [1]. Glycerol and fatty acid are widely used as raw materials, and monoacylglycerol is used as an emulsifying agent in the food, cosmetic and pharmaceutical industries [2]. Recently, diacylglycerol has received much attention as a healthy cooking oil because it has a biological activity to prevent the accumulation of body fat and to lower the level of cholesterol in the blood [3-8].

At present, the Colgate-Emery method has been industrially used for the hydrolysis of triglycerides [9]. This process utilizes steam of high-temperature (523 K) and high-pressure (5.00×10^6 Pa), resulting in high energy consumption and thermal damage of the products. Recently, a hydrolysis method using lipase has been proposed instead of the Colgate-Emery method [10,11]. The enzymatic hydrolysis is conducted under mild condition (at room temperature and atmospheric pressure). Therefore, the above problems can be overcome by this method. Furthermore, the enzymes have substrate and positional specificities [12-17], so that the side reactions such as saponification, polymerization and oxidation are prevented to enhance the yield of the desired product.

In the triglyceride hydrolysis using lipase, the substrates are oil and water, and the hydrolysis takes place at the oil-water interface. In order to industrially utilize this reaction, it is important to elucidate the following subjects.

1) Screening lipases having a high activity.
2) Selecting solvents never lowering the enzyme activity.
3) Investigating effects of various operating factors on the hydrolysis behavior.
4) Constructing kinetic model for enzymatic hydrolysis.

2. Present Status of the Kinetic Model

A large number of studies have been made on the enzymatic hydrolysis of triglycerides. Biochemical studies on screening lipases from various origins were sufficiently conducted, so that their characteristics such as hydrolysis activity and substrate/positional specificity have been clearly understood [12-23]. Several organic solvents never lowering the enzyme activity have been also reported [20-24]. The effects of the operating factors such as temperature, pH and concentrations of enzyme and substrate on the hydrolysis behavior have been experimentally investigated [18-49]. Many kinetic models have been proposed [50-71], but those simplified models were still not enough to describe the complicated mechanism of the enzymatic triglyceride hydrolysis under wide range of operating conditions.

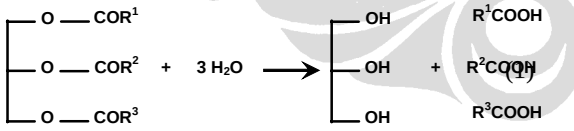
This is because differences between the interfacial and bulk concentrations of the enzyme, substrates and products and the interfacial enzymatic reaction mechanism were not rigorously considered in the models.

3. Differences between the interfacial and bulk concentrations

In order to describe the differences between the interfacial and bulk concentrations of the enzyme, substrates and products, linear/nonlinear relationship were incorporated. The enzyme concentration at the interface was initially assumed to be proportional to that in the bulk phase [59-67]. However, this assumption was not applicable to a high enzyme concentration [68]. Saturated enzyme concentration was reported to be reached at high enzyme concentration [39,62,64]. Thus, a nonlinear relation, such as the Langmuir adsorption model, should be introduced. On the other hand, for the substrates and/or products, the linear relationships between the interfacial and bulk concentrations were usually incorporated [63-68]. This is because the molecular sizes of the substrates and products are much smaller than that of the enzyme, so that the interfacial concentrations do not reach saturation.

4. Interfacial Reaction Mechanism

In order to describe the interfacial enzymatic reaction, reaction mechanisms such as first order, Michaelist-Menten and Ping Pong Bi Bi mechanism were proposed. In the triglyceride hydrolysis, one mole triglyceride (T) reacts with three moles water (W) to produce one mole glycerol (G) and three moles fatty acids (P) as shown by Eq. (1).



In more detail, the triglyceride is stepwise hydrolyzed by the enzyme to be diglyceride (D), monoglycereide (M) and glycerol (G) while the fatty acid is released at each reaction step. The enzyme-substrate complexes are formed at the respective steps. In the simplest model, however, the formation of the enzyme-substrate complexes was neglected, and the irreversible first order reaction mechanism as shown by Eq. (2) was considered [21,50].

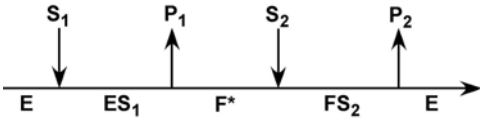
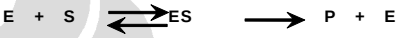


Figure 1. Schematic diagram of Ping Pong Bi Bi mechanism

(2)

In the models considering the formation of the enzyme-substrate complexes, Michaelis-Menten mechanism as shown by Eq. (3) was incorporated[51)- 68)].



(3)

The substrate, S, reacts with the enzyme, E, to form enzyme-substrate complex, ES. Then, product, P, is released. Since one substrate and one product are considered in this mechanism, one fatty acid residue of triglyceride and free fatty acid were simply assumed to be a substrate and a product, respectively.

Recently, there were a few models[69)-71)] incorporating Ping Pong Bi Bi mechanism with two substrates and two products as schematically shown in Fig.1. The reaction proceeds from left to right side as shown by horizontal arrow. The free enzyme, E, reacts with first substrate, S1, to form the first complex, ES1. The first product, P1, is then released from ES1 to form the second complex, F. This complex reacts with second substrate, S2, to form the third complex, FS2. Finally, the second product, P2, is released and the free enzyme is reformed. In case of triglyceride hydrolysis, the first and second substrates were assumed to be one fatty acid residue of triglyceride and water, respectively, while the first and second products were one alcohol residue of triglyceride and free fatty acid, respectively. Triglyceride is stepwise hydrolyzed by the enzyme to be diglyceride, monoglycereide and glycerol, and three ester bonds of triglyceride are not evenly catalyzed by lipase. Although some researchers reported the produced fatty acid inhibited the hydrolysis [20,72,73] the inhibition by fatty acid has never been incorporated in the models considering Ping Pong Bi Bi mechanism.

5. Summary of the Kinetic Model

The proposed models are categorized based on the assumptions for the interfacial reaction mechanism and the differences between the interfacial and bulk concentrations as shown in Figure 2.

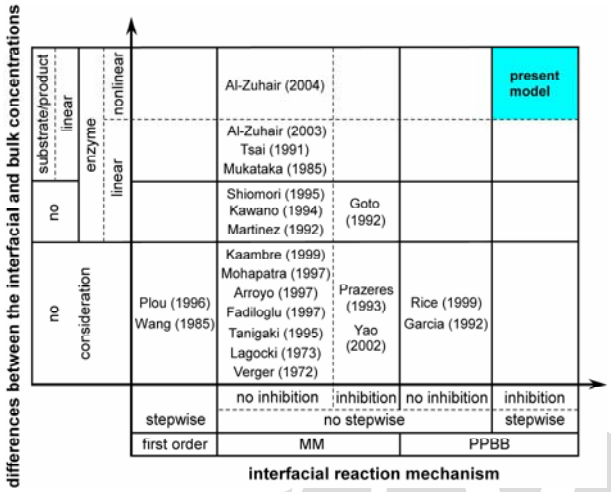


Figure 2. Summary of the kinetic models for enzymatic triglyceride hydrolysis

The assumptions listed as abscissa are first order, Michaelis-Menten (MM) and Ping Pong Bi Bi (PPBB) mechanisms with/without stepwise reaction and/or inhibition by fatty acid and they become more complicated far to the right. The assumptions listed as ordinate are no consideration for the relationship between interfacial and bulk concentration, the linear relationship for substrate/product concentration and the linear/nonlinear relationship for the enzyme concentration and the combination of those relationships also become more complicated upward. In the models without considering the differences between the interfacial and bulk concentrations, the complicated PPBB mechanism has been incorporated by Garcia et al. [69] and Rice et al.[70]. In the models considering the differences between the interfacial and bulk concentrations for not only the enzyme concentration but also the substrate/product concentration, however, only the simple MM mechanism was found to be incorporated. In order to construct a rigorous kinetic model to describe the complicated enzymatic hydrolysis of triglyceride under wide range of operating conditions, therefore, the complicated PPBB mechanism should be considered in addition to the relationships between the interfacial and bulk concentrations used in the Al-Zuhair's model [68]. Furthermore, it is important that the stepwise reaction and the inhibition by fatty acid are taken into consideration in the PPBB mechanism.

The most rigorous kinetic model considering the difference between the interfacial and bulk concentrations of the enzyme, substrates and products, and the interfacial enzymatic reaction mechanism was proposed in this model [71]. The model describing the stepwise hydrolysis of triglyceride by nonspecific lipase in the biphasic oil-water system was formulated on the basis of the following assumptions:

1. Nonlinear relationship between the interfacial and bulk concentrations of the enzyme
2. Linear relationship between the interfacial and bulk concentrations of the substrates and products
3. Stepwise hydrolysis proceeds via a Ping Pong Bi Bi mechanism
4. The inhibition by oleic acid follows the competitive inhibition mechanism
5. The non specific lipase evenly cleave the ester bonds at the edge and the center of the glycerol backbone of the substrates (tri-, di- or monoglyceride)

The model well described the hydrolysis behavior under wide range of operating conditions using Candida rugosa lipase, a nonspecific lipase.

6. Conclusion

In order to construct a rigorous kinetic model to describe the complicated enzymatic hydrolysis of triglyceride under wide range of operating conditions, the difference between the interfacial and bulk concentrations of the enzyme, substrates and products, and the interfacial enzymatic reaction mechanism should be considered in the model.

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