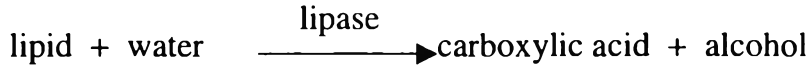
CHAPTER II
LITERTURE SURVEY

2.1 The Function of Lipases as an Enzyme

Enzymes are known as the chemical catalyst controlling all biochemical reactions in the molecular level that plays an important role to induce and storing the energy for living cells. Generally, enzymes are categorized depending on the functions to be:

1. Oxidoreductases-for catalyzation of the oxidation/reduction reactions
2. Transferases-for catalyzation the group transfer reactions
3. Hydrolases-for catalyzation the hydrolytic reactions
4. Lypases-for catalyzation the reactions involving the removal/ addition of a double bond
5. Isomerases-for catalyzation the reactions of isomerization
6. Synthases-for catalyzation the reactions involving the molecules coupling/ breakdown of pyrophosphate of adenosine triphosphate (ATP)

Among the reactions of enzymes, lipase performs a reaction of hydrolyses which can act on the ester bonds, carboxylic esters and glycosyl compounds. The catalytic system of lipase, normally, happens on the water-lipid interface at room temperature by encapsulation the substrate on the specific site which is known as a metabolism of lipid. The reaction can be simplified as shown in Scheme 2.1



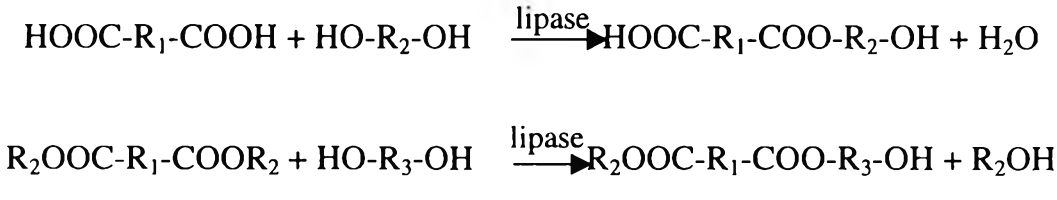
Scheme 2.1 Lipase acts as a catalyst in lipid metablism

The hydrolyses also perform as a reverse reaction of which the lipid will be formed by carboxylic acid and alcohol substrates.

2.2 Lipase for the Artificial Applications

Lipase has received much attention as a catalyst in the reaction owing to the advantages of the reaction that proceeds at room temperature in mild condition including the specific controlled structure of the product obtained. Lipase is reported in the application of the synthesis of ester isomer lipid modification, additive for diesel fuel and Canola hydraulic oil (Seppala *et al.*, 1996).

The biomimetic system using lipase in the artificial reactions has received many interests as referred to its biocatalyst functions on the esterification and transesterification (Seppala *et al.*, 1996). as shown in Scheme 2.2



Scheme 2.2 Lipase catalyst ester synthesis

Thus, lipase shows a potential to be a catalyst for esterification giving polyester from the mild condition with a controlled stereospecific structure.

The development of the biodegradable polyesters concerned to be one of the polymer products in the next century.

2.3 Lipase Catalyst Polyester Synthesis

2.3.1 An Approach for Biodegradable Polymer

Recently, it has been known that biodegradable polymer can be achieved when the structure of the polymer can be well consumed by the digestion of the microbial as seen in the case of poly(hydroxyalkanoate)s series (Steinbuchel *et al.*, 1995). Although various types of biodegradable polymers have been reported, especially the successful of using bacteria *Ralstonia eutropha* to produce poly(3-hydroxybutyrate) (Leigh *et al.*,1998) the practical industries scale is still the problem.

An alternative approach to achieve biodegradable polymer can be mentioned as a controlled structure of polymer synthesis to obtain a particular stereospecific structure of polymer that can be digested by microbial. Conventionally, almost all polymers are synthesized at high temperature under pressure and acid/ base/ metal catalyst in the reaction in order to achieve the high molecular weight. Thus, it is a main point to proceed the reaction without changing any of stereoregularity of the product of which starting from a particular isomer monomer. In such case, the mild condition should be required.

In the case of polyester synthesis, lipase is found to be attractive and shows the potential for the industrial scale. The application of lipase can be expected for not only the biopolymer products, but also the avoid of using toxic catalyst with a consumed energy of severe conditions.

2.3.2 The History and Development

Lipase catalyst polyester synthesis was first reported in 1984, when Okumura *et al.* found that *Aspergillus niger* can be a catalyst for various diacids and diols to give oligoester with a molecular weight about 5,000. In 1985, Ajima *et al.* reported that *Pseudomonas fluorescens* catalyze 10-hydroxydecanoic acid in benzene to obtain an oligoester with molecular weight of 6,000. Although the biomechanism of the lipase in the system is not clear, many lipases with the optimum conditions to obtain polyester products are studied. Wallace *et al.* (1989) is the first group to clarify the porcine pancreatic lipase as a catalyst in the reaction of bis(2,2,2,-trichloroethyl) adipate with 1,4 butanediol to obtain a polymer with molecular weight 10,000-15,000. The reaction was reported to be dependent with the exclusion of the water in the system including the temperature. Seppala *et al.* (1996) summarized 25 lipases to identify the requirement of the lipase for obtaining a high molecular weight product. *Rhizomucor miehei* was found to give a polyester in the molecular weight range of 980-70,430 from the acids of succinic, adipic, octandioic, and sebacic with ethanediol, propanediol, butanediol, pentanediol, and hexanediol. By comparing two types of lipases, i.e., *Rhizomucor miehei* and *Pseudomonas fluorescens*, Seppala *et al.* concluded that the reaction proceeds to obtain a high molecular weight polyester when the active site of the lipase is shallow as seen in the case of *Rhizomucor miehei* (Figure 2.1, 2.2) (Seppala *et al.*, 1996). In 1995, Seppala *et al.* presented that a biodegradable polymer product can be produced owing to the high molecular weight of polyester produced with *Rhizomucor miehei* as a catalyst as seen in the reaction of adipic acid and hexanediol to give 70,000 g/mol polyester. Recently, the application of *Rhizomucor miehei* and porcine pancreatic lipase are received much interest to give a considerable high molecular polyester. The molecular weight can also be covered by using polytransesterification as reported by Brazwell *et al.* (1995), Linko *et al.*

(1995), and Wallace *et al.* (1998) The using of lipase as a catalyst is also expanding to the ring opening polyester synthesis. Bisht *et al.* (1997) proposed the bulk polymerization of ω-pentadecalactone with Novozyme-35 as a catalyst to obtain an oligoester.

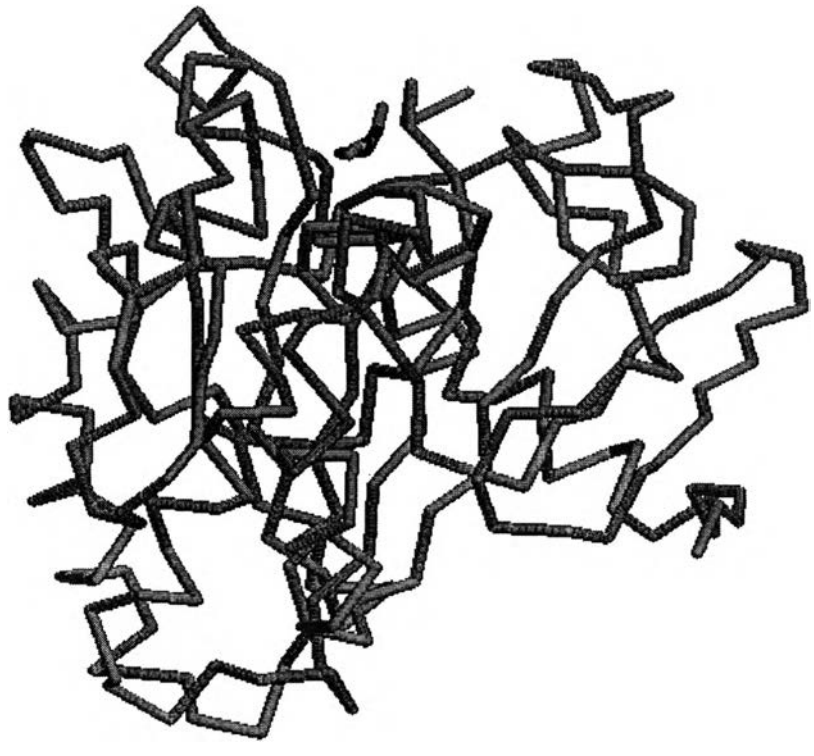


Figure 2.1 Structure of lipase from *Rhizomucor miehei* binding with substrate (PDB entry 5TGL).

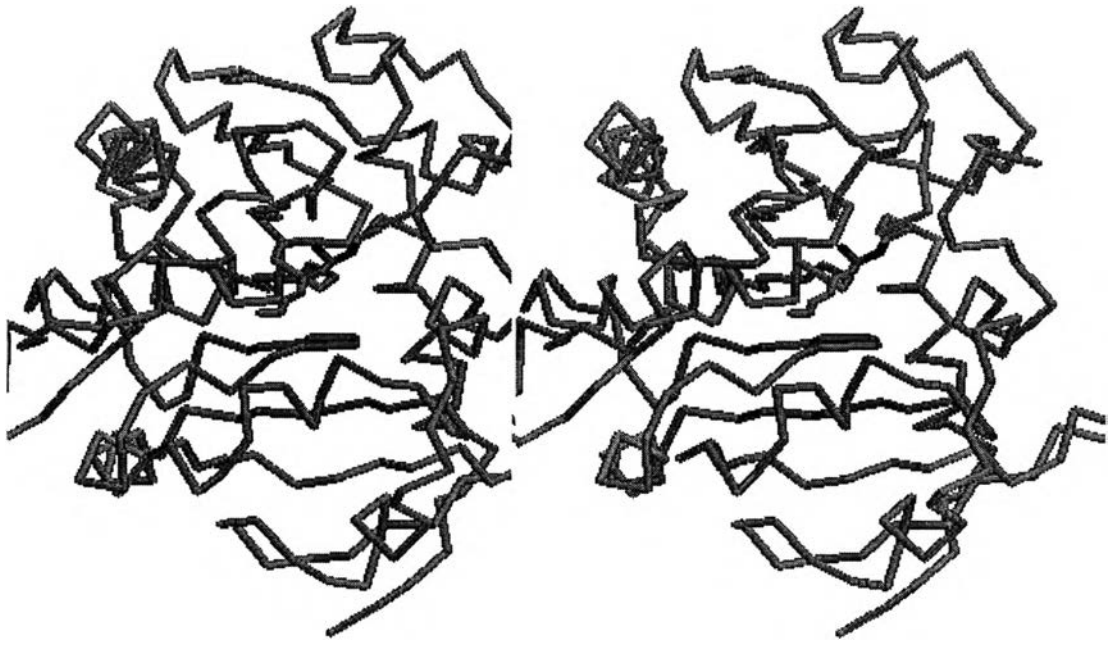


Figure 2.2 Three dimension image of lipase from *Rhizomucor miehei* binding with substrate (PDB entry 5TGL).

2.4 Rice Bran Lipase as an Enzyme Catalyst Polyester Synthesis

The efficiency of lipase as a catalyst for polyester synthesis is known to be related to the active site. Although various types of lipases and the functions have been studied by biochemist researchers, in most cases, the structure of lipase including the active binding site are still not known. The structural characterization of lipase is difficult because it has to be dealt with the formation of crystal and single crystal analysis. Thus, the application of lipase for artificial polymerization has to be based on the discovery the function of the various lipases. Most of the reported lipases are concerned with the lipase from the animal, fungus, and bacteria. However, plants are the sources for lipase and can be expected for the activities that can not be found in animals.

Rice bran lipase is known for its high activity and the stability to digest rice bran oil as always seen in the storing step of rice bran oil production. Funatsu *et al.* (1971) reported the extraction of rice bran lipase from rice bran to find that *japonica* rice bran lipase has the specific activity for 4704.1 mU/mg with a thermal stability at 40°C. Considering the basic property of rice bran lipase, it can be expected that rice bran lipase shows a potential activity to catalyze for the formation of ester.

2.5 Thai Rice Bran Lipase and the Potential of the Present work

Up to now, the lipases reported to be a catalyst polyester synthesis found their own problems about the cost performance including the amount and stability in the ambient temperature.

Thailand is a world top country produces rice. Thus, it is our interest to stand on the viewpoint of using the abundant local product to apply for a unique research. The potential of the work can be claimed not only for the activity and stability of the lipase itself, but also for the overcoming the high cost of the extracted lipases as seen in the case of porcine pancreatic lipase, *Rhizomucor miehei*, *Candida Rugosa*, *Aspergillus niger*, etc.

Although the extraction method of Thai rice bran lipase has not been standardized and reported, the present work will cover the study on the extraction process to obtain the most stable lipase with the high specific activity. The present work will also study on the Thai rice bran lipase for the possibility as a catalyst in esterification and polyesterification.