ต้นฉบับ หน้าขาดหาย

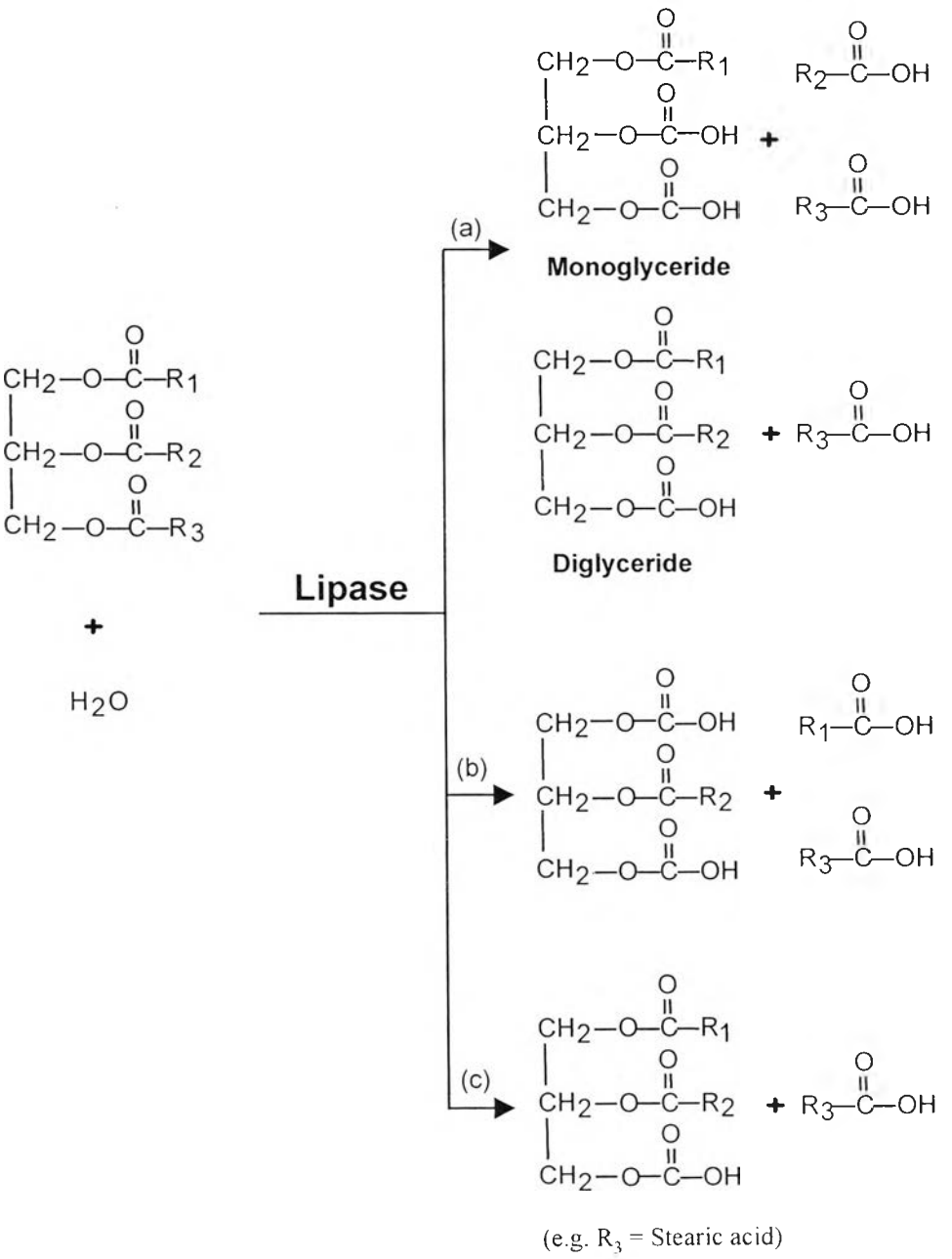
2.2 Lipase-Catalysed Polyesterification

Lipases or triacylglycerol acylhydrolases (EC 3.1.1.3) are enzymes that catalyze the hydrolysis of long-chain fatty acid triacylglycerols to free fatty acids and glycerols (Scheme 2.1). The most common sources of lipases are microorganisms (e.g., fungus, yeast, bacteria) followed by mammalian cells (e.g., pig pancreases, horse pancreases) and plants (e.g., wheat germ, rice bran). Lipases can be categorized into three groups, i.e., nonspecific lipases, 1,3-specific lipases, and fatty acid-specific lipases, based on their specificity (Mukherjee and Hills, 1994). Nonspecific lipases break down triglyceride molecules at random positions, giving free fatty acids and glycerol with monoglycerols and diacylglyderols as products. 1,3-specific lipases catalyse the hydrolysis of fatty acids at positions 1 and 3 of glycerol backbone. With fatty acid-specific lipases, only particular type of fatty acids is released from triglyceride molecules (Macrae, 1983).

In addition to hydrolysis, some lipases are stable in organic solvents and can be used as biocatalyst for esterification, transesterification and polyesterification. Lipase-catalysed synthesis has become a promising methodology for polyester synthesis (Okumura, 1984) since it offers many potential advantages in terms of stereoselectivity, high catalytic activity, lack of undesirable side-reactions and operations under mild conditions.

Lipase-catalysed 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 molecular weight about 5 000. Wallace *et al.* (1989) is the first group to obtain high molecular weight polyester of 10 000-12 000 from using porcine pancreatic lipase in transesterification of bis(2,2,2-trichlotoethyl) adipate with 1,4-butanediol. The reaction was reported to be dependent with exclusion of the water in the system. Linko *et al.* (1995) presented that the removal of by-product leads to

Scheme 2.1 Triglyceride hydrolysis by lipase at the position of a) nonspecific, b) 1,3-specific, and c) fatty acid-specific



high molecular weight polyester (46 400) obtained from polyesterification of sebacic acid and 1,4-butanediol, having *Rhiomucor miehei* lipase as a catalyst. In addition to polytransesterification and removal of by-product, high molecular weight polyester is also obtained by using irreversible acyl donor as monomer. Uyama and Kobayashi (1993) used divinyl adipate to react with 1,4-butanediol under *Pseudonomas fluorescens* lipase catalytic system to obtain polyester with the molecular weight 12 000.

2.3 Lipase Immobilization

The practical applications of lipases in industrial scale can be raised as detergent, cosmetic, textile, and food manufacturing. However, one of the main hindrances for industrial application of biocatalysis is the ease of lipase deactivation when they are exposed to heat and/or extreme acid or base condition. Increasing lipase thermal stability allows enzymatic reactions to be carried out at higher temperatures, which helps increasing conversion rate, substrate solubility and reducing the possibility of microbial growth and the viscosity of the reaction medium. Many researches have been focused on the enhancement of lipase stability at high temperatures by various strategies, e.g., the use of soluble additive in protein engineering, chemical modification and immobilization.

Lipases are immobilized by many procedures that can be categorized to three major methods, i.e., binding of the enzyme with a solid carrier by physisorption (van der Waals, hydrophobic interaction or ionic bond) and chemisorption or covalent bond, cross-linking with the help of bifunctional agents, and entrapment of the enzyme in gel, capsule or membrane reactors.

Immobilization of lipases by binding onto solid carriers offers several advantages, including the simple recovery which allows the reuse of the catalyst, the easy separation of enzyme from product, the possibility of

continuous operation in a packed-bed reactor, and the improvement of enzyme activity and stability.

Physisorption is the most common immobilization procedure because of its simplicity and low cost. However, its major drawback is that the immobilized enzymes are easily desorbed from the carrier during the utilization. Due to this problem, chemisorption or covalent bonding is proposed as an alternative way. Covalent attachment is the popular one of immobilization strategies because of the strongly bound onto carriers and the vareity of immobilization. Although covalently immobilized enzymes provide lower activity but its stability is improved when compared to that from physisorption (Arroyo, 1999). Brady *et al.* (1988) searched for adsorbents to be suitable supports for lipase. Adsorbents, such as celite, cellulose, ethyl cellulose, silica gel, kieselguhr, clay, alumina, accurel, celgard 2500, CPG-100, carbon, profax PP, and microtene HDPE, etc., were chosen as possible immobilization supports for *Candida rugosa* lipase. Most of these supports were found to decrease the lipase activity significantly upon immobilization. The hydrophobic microporous materials, such as accurel and celgard 2500, are known to give good performances. Coa *et al.* (1998) studied the immobilized lipase from *Candida antarctica B* onto various supports, such as celite, Hyflo Super Cel, Amberlite XAD-7 and polyprolylene (EP 100), to find that polypropylene (EP 100) showed the highest hydrolytic activity with the thermal stability improvement.

The effect of pore size on lipase immobilization was also the factor to be discussed. Shaw *et al.* (1989) used cross-linked sepharose with different pore sizes as supports for *Candida rugosa* lipase. It was found that the smaller pore size provided the poorer accessibility of the substrate into, leading to low activity.

The reaction media is known to affect both the conformation and the relative reactivity of the functional groups of protein. As a result, it

influences the immobilization pattern. Due to more interactions between enzyme and support or chemical functional group, which can lead to the negative effect on enzyme performance, the spacer is proposed as a method to avoid the problem. Stark and Holmberg (1989) found that the activity of the immobilized lipase from *Rhizopus* prepared in hexane was higher than that in buffer while longer hydrophilic spacer had a larger effect on activity in hydrolysis reaction, but no effect on activity in transesterification.

Pretreatment of the support with organic solvents also affects significantly to the immobilized lipase activity. Montero *et al.* (1993) pretreated polypropylene carrier with pure organic solvents, such as ethanol, acetone, acetonitrile, 2-propanol or methanol, and found that it led to high adsorption and immobilized activity.

2.4 Immobilized Lipase-Catalysed Polyesterification

Immobilized enzymes are used in organic synthesis to fully exploit the technical and economical advantages of biocatalyst. Immobilized enzymes bring a more convenient handling of catalyst reaction, which are the easy separation of the enzyme out of the product, and the reuse of the enzyme. The easy separation of the enzyme from the product simplifies enzyme applications and offers the purity of product. On the other hand, the ability to be reused of enzymes provides cost advantages. In some cases, enzyme immobilization offers the improvement of enzyme activity and stability.

According to these advantages, immobilized lipase is an alternative way to improve the enzyme-catalysed polyester synthesis. Uyama *et al.* (1997) applied immobilized lipase derived from *Candida antarctica* in the ring-opening polymerization of lactones, ε-caprolactone, 11-undecanolide, 12-dodecanlide. Only small amount of the immobilized lipase (1% weight of monomer) showed the extremely efficient catalysis in the lactone

polymerization, comparing to those by powdery lipase (20-50% weight of monomer). The molecular weight of the obtained polyester from the ring-opening polymerization of lactones, ε-caprolactone, 11-undecanolide, 12-dodecanlide are 5 200, 5 500, and 3 400, respectively. Mezoul *et al.* (1995) reported enzyme-catalysed synthesis of aliphatic polyester from methyl diesters and diols in the presence of lipozyme (*Mucor miehei* lipase immobilized on a macroporous anion exchange resin) and novozyme (*Candida antarctica* lipase immobilized on macroporous acrylic resin). Pencreach *et al.* (1997) found that the fatty acid specificity of the immobilized lipase was changed comparing to free lipase. The carbon chain length was varied from 2 to 18. After immobilization of *Pseudomonas cepacia* lipase on microporous polypropylene (Accurel EP-100), the lipase was mainly specific for short chain fatty acid esters (carbon chain length < 8), whereas the free enzyme was mainly specific for long chain esters (carbon chain length > 8). The thermal stability of immobilized lipase at 80°C was twice as much as free lipase.

2.5 Rice Bran Lipase-Catalysed Polyesterification

Rice is one of the world's important cereals serving as a food for a large number of people in the world. From rice production, rice bran is obtained as by-product, which is a rich source of lipids, vitamins, mineral, proteins, and numerous enzymes, including lipase. Rice bran lipase is known for its high activity and the stability. Rice bran lipase even digests rice bran oil, which is concerned to be one of the problems in the storing of rice bran oil production. By considering this basic property, it can be expected that rice bran lipase shows a potential activity on ester formation. However, rice bran lipase is rarely applied as biocatalyst in ester and polyester synthesis. Phraephrewngarm (1999) studied the preparation of Thai rice bran lipase and

its possibility to be a catalyst in polyester synthesis to find that oligoester with MW 1 022 was obtained from polyesterification of adipic acid and 1,4-butanediol.

2.6 The Scope of the Present Work

Thailand is a world top country to produce rice. Thus, it is our interest to stand on the viewpoint of using the abundant local product to apply for a unique research. Moreover, our recent work clarified rice bran lipase for its activity on polyester synthesis (Phraephrewngarm, 1999). The present work was based on the development of the system for practical application, the reactivity and stability of the Thai RBL. The strategy to obtain the polymer chain was designed by applying the stepwise reactions of monomer-monomer and monomer-polymer under Thai RBL catalytic system. The unique of the present work was to demonstrate the polymerization of polyol and diacid by Thai RBL catalytic system. The present work also concerned the practical application of Thai RBL by studying immobilization onto carrier, such as fumed silica, alumina, and celite, via physisorption and covalent bonding. The activity improvement of the immobilized lipase was further considered by enzyme purification approach.