Enzymatic Polymer Synthesis: An Opportunity for Green Polymer Chemistry

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1. Introduction

Polymeric materials, natural and unnatural, are indispensable to the modern society. They are widely used from everyday life usages as commodity materials to industry and technology usages in the fields such as electronics, machinery, communications, transportations, pharmacy, and medicine as highly advanced materials. Today, it is hard to think of the present society without polymeric materials.

Developments of various polymeric materials have been owed to epoch-making innovative works as exemplified typically by the discovery of Ziegler−Natta catalyst,[1−3] the concept of living polymerization,[4] the discovery of conducting polymers,[5] and the discovery of metathesis catalyst.[6,7] These observations demonstrate that new polymeric materials are often brought about by new production methods including polymerization catalysts. Historically, polymerization catalysts utilized classical catalysts of acids (Brønsted acids, Lewis acids, and various cations), bases (Lewis bases and various anions), and radical generating compounds since the 1920s, the early stage of polymer chemistry. In the following

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Shiro Kobayashi studied organic chemistry and polymer chemistry in Kyoto University and received his PhD in 1969. Then, he stayed at Case Western Reserve University as a postdoc for two years. In 1972, he joined Kyoto University as a research associate and started to investigate polymer synthesis. He then joined at Mainz University as a Humboldt fellow in 1976. Following a lectureship in Kyoto University, he was appointed as a full professor of Tohoku University in 1986 and started research on enzymatic polymerization. He moved to Kyoto University in 1997 and officially retired in 2005 to become a professor emeritus. Since then he has been a distinguished professor at Kyoto Institute of Technology. His research interests include polymer synthesis, organic reactions, material science and, in particular, enzymatic polymerization, which enabled the first chemical synthesis of various natural and unnatural polysaccharides, functionalized polyesters and phenolic polymers. He received the Chemical Society of Japan Award for Young Chemists (1976), the Award of the Society of Polymer Science Japan (1987), the Humboldt Research Award (1999), the Chemical Society of Japan Award (2001), the John Stauffer Distinguished Lecture Award (2002), and the Medal with Purple Ribbon (2007) among others. He is a foreign member of the Northrhine Westfalian Academy of Science since 1999. He currently serves as a member of (executive) advisory board and editorial (advisory) board for fourteen international journals.

Akira Makino was born in Sapporo, Japan, in 1977. He studied macromolecular chemistry at Kyoto University and received his degrees of B.Sc. in 2001 and M.Sc. in 2003. In 2006, he obtained his Ph.D. in engineering from Kyoto University under the supervision of Prof. Shiro Kobayashi and Prof. Shunsaku Kimura. He started his postdoctoral research carrier at Shimadzu Corporation, Japan. In 2007, he joined the Department of Material Chemistry, Kyoto University, as an assistant professor. His current research interests focus on the synthesis of biocompatible functional materials based on macromolecular chemistry.

stage, the catalysts started to use the transition metals in Ziegler−Natta catalyst in the 1950s and later in the metathesis catalyst, as well as using the rare-earth metals in these catalysts. These catalysts still have major roles in the polymer synthesis.

Since two decades earlier, however, a new approach of polymer synthesis has been developed, employing enzymes as catalysts (enzymatic polymerization),[8] although in vitro enzymatic catalysis has been extensively used in the organic synthesis area as a convenient and powerful tool.[9−13] Moreover, an advanced technique of catalysis in the organic synthesis enabled control of the chirality problem for the production of only desired molecules selectively via asymmetric reactions.[14] However, polymerization needs many repetitions of a propagation reaction that must be "selective" in all respects for producing a polymer molecule of high molecular weight.

Enzymes are biocatalysts, which catalyze all metabolic reactions in vivo for maintaining "living life". The biocatalysts generally include enzymes, microorganisms, and even higher organisms; however, this article focuses on the in vitro enzymatic catalysis to produce polymeric materials, and hence, in vivo polymer synthesis such as a fermentation process and a synthesis method using *Escherichia coli* technology are not included. Thus, *enzymatic polymer synthesis*, which includes *enzymatic polymerization* and *enzymatic polymer modification*, is discussed. Enzymatic polymerization is defined as "the in vitro polymerization of artificial substrate monomers catalyzed by an isolated enzyme via nonbiosynthetic (nonmetabolic) pathways".[8,15−18] In these two decades in fact, many enzymatic polymerization reactions have been developed, and a variety of new polymers have been created via enzymatic polymerization, which relates to sections 3−7.[8,15−23] Enzymatic polymer modification denotes a "modification reaction of the existing polymers with enzymatic catalysis". This is another effective method to produce new polymeric materials because of the selective catalysis, which relates to section 8.[8,22,23] In vitro enzymatic catalysis in synthesizing polymers holds the characteristics of in vivo enzymatic catalysis and, in particular, reaction selectivity. Almost of all the reactions proceed in a selective way with precise structure control and involve the green nature of the reaction with various respects. Accordingly, "enzymatic polymer synthesis" provides an opportunity to conduct "green polymer chemistry".[8,17,18,20,22,24,25]

2. Enzymes and Enzymatic Reactions

All of the biopolymers (biomacromolecules, Figure 1) are produced in living cells with enzymatic catalysis. Since the first discovery of the enzyme of diastase (amylase) by A. Payen and J. Persoz in 1833, enzymatic catalysis has been mysterious yet attractive to scientists in many fields. Research on finding new enzymes and on the mechanisms of enzymatic reactions has been among the most important central topics in the fields such as biochemistry, organic chemistry, medicinal chemistry, pharmaceutical chemistry, and polymer chemistry.[26]

All enzymes are classified into six main groups according to the Enzyme Commission (Table 1). Nowadays, many thousands of enzymes are commercially available and some of them are mutated for industrial applications. Generally, oxidoreductases, hydrolases, and isomerases are relatively stable, and some of isolated enzymes among them are conveniently used as catalysts in a practical manner, as in the chemical, food, and pharmaceutical industries. In contrast, lyases and ligases are present in lesser amounts in living cells and are less stable for isolation or separation from living organisms. So far, three groups of enzymes have been employed as catalyst for enzymatic polymerization. For enzymatic polymer modification, four enzyme groups have been applied as catalysts.

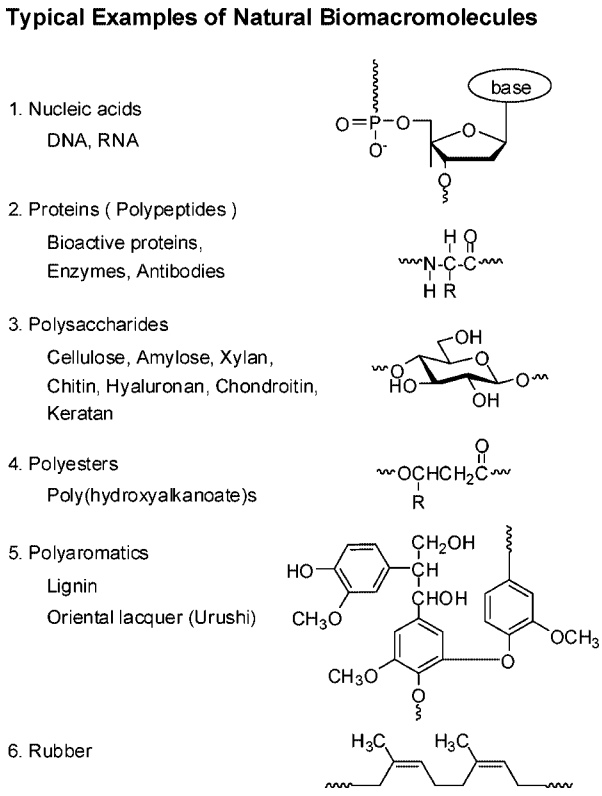


Figure 1. Typical examples of natural biomacromolecules and their structures, simply depicted.

As to in vivo enzymatic catalysis, various characteristics have been revealed through a large number of studies over the century. Among them, the following two fundamentally important characteristics are to be mentioned because they are deeply conceptually related to the development of enzymatic polymerization. The first is a "key and lock" theory proposed by E. Fischer in 1894, which pointed out the relationship between an enzyme and a (natural) substrate.[27] The theory implies that the enzyme catalyzes a reaction of only the specific substrate that is recognized by the enzyme like a key and lock relationship as shown simply in the upper cycle (A) in Figure 2.[28] In the enzyme−substrate complex, a substrate (key) is located in the enzyme (lock) with geometrical adaptation, and then, the substrate is activated and the bond formation is accelerated to lead to the product.

The second is again concerning the reaction mechanism. L. Pauling suggested the reason why an enzymatic reaction progresses under mild reaction conditions.[29,30] The activation energy is much lowered by stabilization of the *transition-state* using an enzyme−substrate complex as compared with that in the no-enzyme case (Figure 3). The rate acceleration is normally 10^6-10^{12} fold; however, a specific case reached even 10^{20} fold![31] The mechanism of in vivo enzymatic reaction shown in Scheme 1 is generally well accepted[32] and corresponds to the reaction coordinate in Figure 3. How is the transition-state $[ES]^{\ddagger}$ formed? What would its structure be? These questions are the key for understanding enzymatic reactions. From the in-depth consideration, in fact, the concept of catalytic antibody was created.[17,33]

The key and lock relationship observed for in vivo reactions is not absolutely strict for all enzymatic reactions. The enzyme is often dynamic and able to interact with not only a natural substrate but also an unnatural substrate. In the in vitro enzymatic polymerization, a monomer is an unnatural (artificial) substrate for the catalyst enzyme. Yet, the substrate is to be recognized and to form an enzyme-artificial substrate complex so that the desired reaction may take place. For this reason, it has been proposed that the monomer is to be designed according to a new concept of a "transition-state analogue substrate" (TSAS), the structure of which should be close to that of the transition-state of the in vivo enzymatic reaction.[8,17,18,20,22,28,34−38] This is because the enzyme stabilizes the transition-state via complex formation with the substrate.[29,30] The artificial monomer thus appropriately designed forms readily an enzyme−substrate complex and the reaction is induced to give the product with liberating the enzyme again as shown in the lower cycle (B) in Figure 2. It is stressed that a structurally close transition-state must be involved commonly in both cycles A and B.

All in vivo enzymatic reactions involve following characteristics: (i) high catalytic activity (high turnover number), (ii) reactions under mild conditions with respect to temperature, pressure, solvent, pH of medium, etc., bringing about energetic efficiency, and (iii) high reaction selectivity of regio-, enantio-, chemo-, and stereoregulations, giving rise to perfectly structure-controlled products. If these in vivo characteristics could be realized for in vitro enzymatic polymer synthesis, we may expect the following outcomes: (1) perfect control of polymer structures, (2) creation of polymers with a new structure, (3) a clean, selective process without forming byproducts, (4) a low loading process with saving energy, and (5) biodegradable properties of product polymers in many cases. These are indicative of "green" nature of the in vitro enzymatic catalysis for developing new polymeric materials. Actually, many of the expectations have been realized as seen in the following sections.

3. Synthesis of Polysaccharides

Polysaccharides belong to one of three major classes of natural biomacromolecules, together with nucleic acids and proteins (Figure 1). Polysaccharides have very complicated structures, having many stereo- and regioisomers. The synthesis of two other major biomacromolecules has been facilitated for many years by utilizing an automated solid-phase synthesizer or a genetic engineering procedure. In contrast, it has been so hard to develop a versatile synthesis method of polysaccharides. This is partly because, unlike the synthesis of proteins in vivo biosynthesis, the polysaccharide synthesis is not under the direct control of the DNA system. Furthermore, it was essential to repeat a precise regio- and stereoselective glycosylation for the preparation of polysaccharides with conventional organic chemistry. Therefore, the synthesis of polysaccharides was far behind that of nucleic acids and proteins. In these two decades, however, a new method of synthesizing structurally well-defined polysaccharides has been developed by using enzymes as catalyst.

The polysaccharide synthesis needs a highly selective glycosylation reaction between a donor molecule and an acceptor molecule to form a glycosidic linkage as exemplified in a simple reaction of $\beta(1\rightarrow4)$ linkage formation (Scheme 2). This type of glycosylation reaction must occur repeatedly many times for producing a high molecular weight polysaccharide.

Enzymes show high reaction specificity and catalyze a particular reaction in a regioselective and stereocontrolled manner. In the biosynthetic pathway of natural polysaccharides, formation of a glycosidic linkage is mainly catalyzed by glycosyltransferases employing the corresponding sugar

Table 1. Classification of Enzymes, Their Examples, Typical Polymers Synthesized by In Vitro Enzymatic Catalysis, and Polymer Modification

	enzymes	example enzymes	polymers synthesized	modification
1.	oxidoreductases	peroxidase, laccase, tyrosinase, glucose oxidase	polyphenols, polyanilines, vinyl polymers	○
2.	transferases	glycosyltransferase, acyltransferase	polysaccharides, cyclic oligosaccharides, polyesters	○
3.	hydrolases	glycosidase (cellulase, amylase, chitinase, hyaluronidase), lipase, peptidase, protease	polysaccharides, polyesters, polycarbonates, polyamides, polyphosphates, polythioesters	○
4.	lyases	decarboxylase, aldolase, dehydratase		
5.	isomerases	racemase, epimerase, isomerase		○
6.	ligases	ligase, synthase, acyl CoA synthetase		

nucleotides as substrate. For example, natural cellulose is synthesized in vivo from uridine-5′-diphospho (UDP)-glucose via cellulose synthase (EC 2.4.1.12)-catalyzed reaction

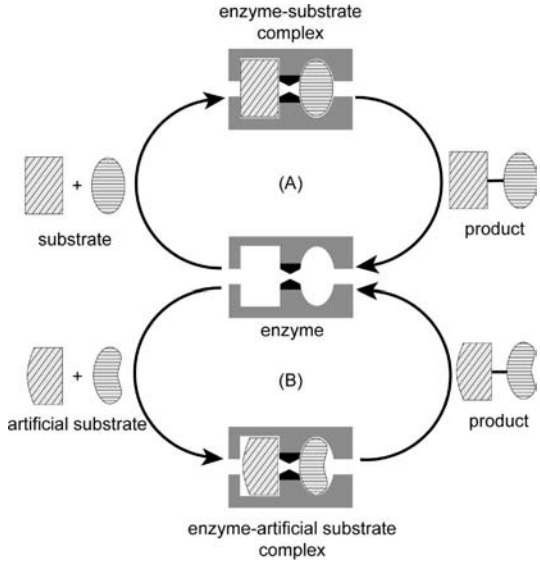


Figure 2. Enzyme−substrate relationship for enzymatic reactions: (A) an in vivo reaction obeying the "key and lock" theory and (B) an in vitro reaction involving an enzyme-artificial substrate complex leading to a product with bond formation, where the artificial substrate desirably possesses a close structure of the natural substrate at the active site vicinity. The black part in the enzyme indicates the active site. (Reprinted with permission from ref 28. (Figure 2). Copyright 2006 Springer.)

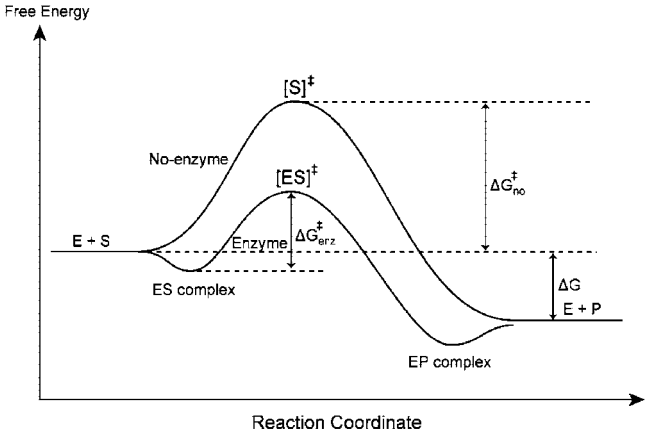


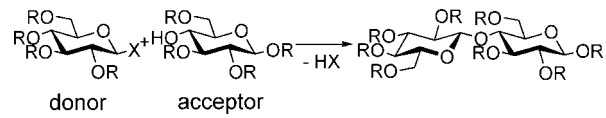
Figure 3. Energy diagram for a chemical reaction: An enzyme-catalyzed reaction proceeds much faster than a no-enzyme reaction, by lowering the activation energy with stabilizing the transition-state of the reaction.

Scheme 1

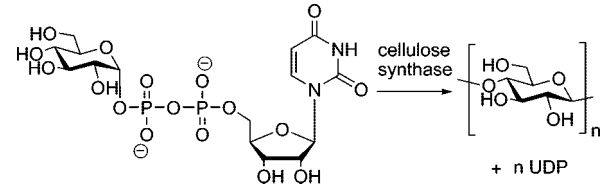
$$E + S \rightleftharpoons ES \rightleftharpoons [ES]^{\ddagger} \rightleftharpoons EP \rightleftharpoons E + P$$

E: enzyme, S: substrate, P: product

Scheme 2



Scheme 3



(Scheme 3).[39,40] However, glycosyltransferases are generally transmembrane-type proteins, present in nature in very small amount, and unstable for isolation and purification with difficulty in handling. Glycosyltransferases are not only expensive, but hardly available. Then, in the late 1980s, research work employing glycoside hydrolases as catalyst was initiated for the in vitro synthesis of polysaccharides (enzymatic polymerization).[8,17,34] Glycoside hydrolase (EC 3.2.1) is a hydrolysis enzyme, which catalyzes cleavage reaction of glycosidic linkages. The enzyme is classified into two groups, endo- and exo-type enzymes. An endo-type glycoside hydrolase, whose shape at the catalytic domain looks like cleft, showed high catalytic activity for the enzymatic polymerization.[8,17,28,34,35,41−43]

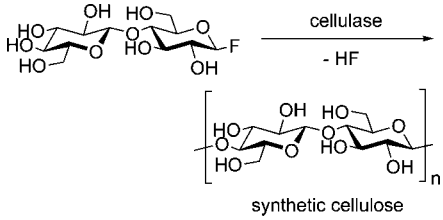
3.1. Natural Polysaccharides and Their Derivatives via Polycondensation

3.1.1. Cellulose and Its Derivatives via Cellulase-Catalyzed Polycondensation

3.1.1.1. Cellulose. Cellulose is a linear polysaccharide composed of $\beta(1\rightarrow4)$ linked D-glucose (Glc) and is, by far, the most abundant organic compound on the earth. It is one of the three major structural components of the primary cell walls of green plants, along with hemicellulose and lignin. From the ancient times, cellulose has been widely utilized as energy source and materials for building and clothing. Still today, cellulose is an important raw, renewable material because of its physically strong, biodegradable, and biocompatible characters. The application area of cellulose is spread to value-added materials in various fields by the chemical modification to functionalized cellulose derivatives.[44]

Since the importance of cellulose much attracted chemists to achieve in vitro chemical synthesis of cellulose, the challenge for this started from an attempted synthesis in 1941.[45] However, the difficulty resulting from repeated glycosylation reactions in a regioselective and stereocontrolled manner has been an obstacle for the synthesis via conventional organic chemistry techniques. In 1991, the first in vitro synthesis of cellulose was reported for the poly-

Scheme 4



merization of β-cellobiosyl fluoride (β-CF) monomer catalyzed by cellulase from *Trichoderma viride* (EC 3.2.1.4).[46−48] Cellulase is an enzyme, which catalyzes the hydrolysis reaction of $\beta(1\rightarrow4)$ glycosidic linkage between two Glc units in vivo. Using an acetonitrile/acetate buffer (pH 5.0) (5:1, v/v) mixed solution as solvent, cellulase catalyzed the bond-formation reaction, and produced "synthetic cellulose" having a perfectly controlled $\beta(1\rightarrow4)$ glycosidic structure with degree of polymerization (DP) value around 22 (Scheme 4). During the reaction, HF was liberated and hence the reaction is of polycondensation.

Proposed mechanism of the cellulose synthesis is illustrated in Figure 4.[8,28,34,41] Family 5 cellulase has a cleft-like catalytic domain. There are two carboxyl acid residues involved in the catalysis.[49] In the hydrolysis, one residue protonates glycosidic oxygen atom, and the other pushes the anomeric (C-1) carbon in the general acid−base mode, facilitating the cleavage of the glycosidic linkage (stage a). Then, a highly reactive intermediate (or transition-state) of a glycosyl−carboxyl structure with α-configuration is formed (stage b). To the anomeric center of the intermediate, a water molecule attacks from the β-side, and hydrolysis is completed (stage c). Therefore, family 5 cellulase is a retaining enzyme with "double displacement mechanism", which involves twice inversions of anomeric stereochemistry. In the polymerization, immediately after the recognition of β-CF monomer at the donor site of cellulase catalyst, fluoride anion is readily eliminated as HF molecule via general acid−base mechanism (stage a′). The monomer forms a glycosyl−carboxyl intermediate with α-configuration, whose structure is regarded as similar with that of stage b of the hydrolysis (stage b′). The C-4 hydroxy group of the disaccharide monomer or the growing chain end located at the acceptor site attacks the anomeric carbon from the β-side, and new $\beta(1\rightarrow4)$ glycosidic linkage is formed (stage c′). Thus, β-CF is considered as a transition-state analogue substrate (TSAS) monomer because of a transition-state (or intermediate) structure involved in common in both reactions from stage a (a′) to stage b (b′). In other words, the transition-state, the structure of which is probably rather close to that of the starting substrate of β-CF, comes early.[17,34,41] During the polymerization, this glycosydic linkage formation reaction is repeated, and hence, the monomer acted as a glycosyl donor as well as a glycosyl acceptor.

The reason why β-CF is considered to be an excellent TSAS monomer is because of the following: (1) the disaccharide structure of cellobiose is the smallest unit of the cellulose repeating unit structure to be recognized by cellulase, (2) the size of fluorine atom (covalent radius, 0.64 Å) is close to that of oxygen (0.66 Å), giving a desired opportunity for β-CF to be recognized by cellulase, (3) the fluoride anion is the best leaving group, and (4) only the glycosyl fluoride is stable among the unprotected glycosyl halides.[8,34,41]

In contrast, under the same reaction conditions α-cellobiosyl fluoride (α-CF) was recovered unchanged from the reaction mixture, indicating that α-CF was not recognized by the enzyme. When β-lactosyl fluoride (β-LF) was used, only hydrolyzed products of the disaccharide β-LF were formed, suggesting that β-LF bearing D-galactose (Gal) was recognized at the donor site, but β-LF was hardly recognized at the acceptor site of the enzyme for the glycosidic linkage formation.[46]

With the use of the cellulase catalyst specificity for substrates, cellooligosaccharides were prepared via a stepwise condensation of sugar fluorides with perfect control of regio- and stereochemistry without the use of protecting groups.[50−53]

Another approach of cellulose synthesis extending the above method in principle was reported, that utilized a disaccharide monomer of cellobiose and a cellulase/surfactant (CS) complex as catalyst.[54] The CS complex was consisted from the mixture of cellulase (*Trichoderma viride*) and a specific nonionic surfactant of dioleyl-*N*-D-glucona-L-glutamate ($2C_{18}\Delta^9$GE). In the nonaqueous medium of dimethylacetamide (DMAc)/LiCl, a cellulose-solubilizing solvent, polymerization took place at 37 °C to give white powders as synthetic cellulose (Scheme 5). The DP value was high (over 100), and the product yield was low (up to 5%). The reaction was of dehydration polycondensation and controlled regioselectivity and stereochemistry, involving extensive transglycosylation of the product revealed from the MALDI-TOF/MS measurement.

In a DMAc/LiCl solvent system, nonreducing end of cellulose, located on the surface of the crystalline structure is considered to be loosened to some extent. By the CS complex-catalyzed transglycosylation of lactose to the cellulose nonreducing end, surface modification of crystalline cellulose could be achieved.[55]

For reference, chemical synthesis of cellulose based on a conventional organic technique was archived in 1996 via cationic ring-opening polymerization of 3,6-di-*O*-benzyl-α-D-glucose 1,2,4-orthopivalate.[56] After removal of the protecting groups from the polymerization product, it was claimed to be synthetic cellulose with a similar DP value with that obtained by the enzymatic method.

3.1.1.2. Cellulose Derivatives. Various kinds of chemically modified cellulose have been synthesized for utilization of cellulose as functional materials. Such cellulose derivatives include industrially utilized methyl cellulose and carboxylmethyl cellulose. However, chemical structure of these cellulose derivatives is not able to be precisely controlled in terms of regioselectivity of the three different hydroxy groups. Enzymatic polymerization allowed to synthesize a cellulose derivative with well-defined structure, since the product structure can be designed by designing the monomer.

The *S*-linked β-cellobiosyl fluoride monomer was polymerized with catalyst of cellulase from *Trichoderma viride* to give oligomers of alternating *O* and *S* linkages. The reaction products gave tetra-, hexa-, octa, and deca-saccharides in 4.5, 7.5, 5.7, and 5.0% isolated yields, respectively.[57]

Syntheses of C-6 methylated cellulose derivatives via cellulase-catalyzed polymerization of 6-*O*-methyl- and 6′-*O*-methyl-cellobiosy fluorides were examined (Scheme 6).[58] 6-*O*-Methyl-β-cellobiosy fluoride was recognized by cellulase as a TSAS, giving rise to the corresponding alternatingly C-6 methylated cellulose derivative in a mixture of acetonitrile and acetate buffer at pH 5.0. The gel permeation chromatographic (GPC) analysis of the acetylated product

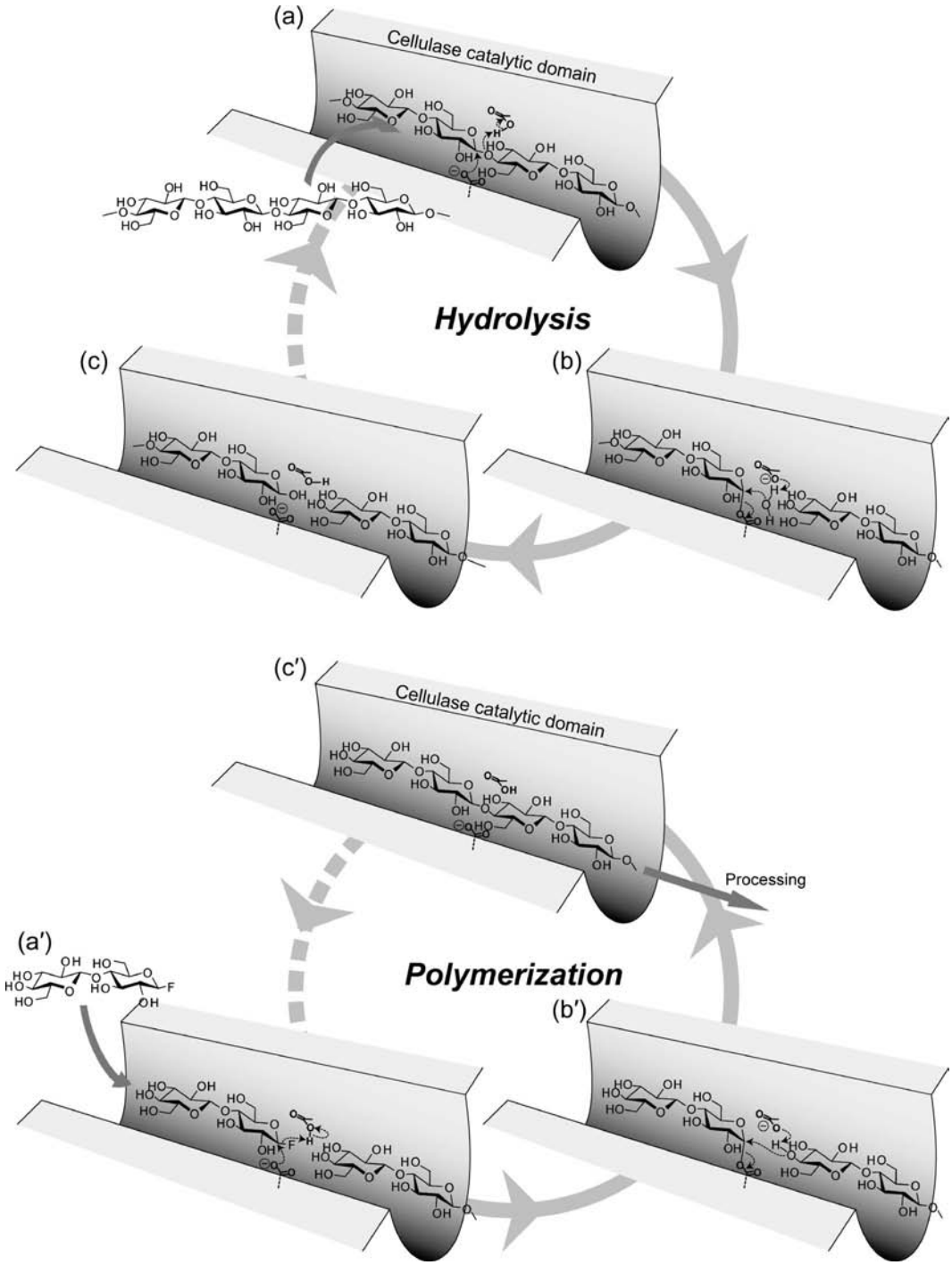
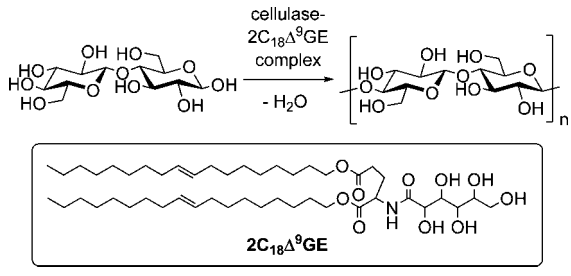


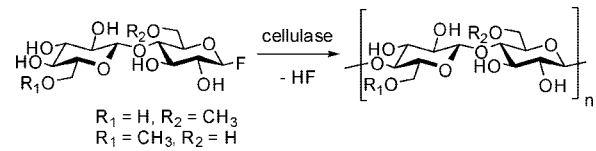
Figure 4. Postulated reaction mechanisms of cellulase catalysis for hydrolysis of cellulose (upper cycle) and for polycondensation of β-CF to synthetic cellulose (lower cycle).

Scheme 5



revealed that the number-average molecular weight (M_n) was 3.9×10^3, corresponding to $n = 7$. On the other hand, only

Scheme 6



the dimer (tetrasaccharide) was obtained from 6′-*O*-methyl-β-cellobiosy fluoride.

3.1.1.3. Mutant Cellulase Catalyst. Enzymatic polymerization using a hydrolase as catalyst accompanies the hydrolysis of the products. To suppress this undesired side reaction, the polymerization was normally conducted in an acetonitrile/buffer mixed solvent.[46,58] The perfect inhibition

of the side reaction was very difficult. Mutation of cellulase, therefore, was variously examined.

There are two approaches reported for the mutation of cellulase: one is to exclude a carboxylic acid group of proton donor or nucleophile in the catalytic domain, and the other is to remove cellulose-binding domain, which is necessary to capture and take crystalline cellulose chain into the catalytic domain. In 1998, glycosidic linkage formation catalyzed by mutated glycosidases was reported.[59,60] The E358A (E = Glu, A = Ala) mutant of exo-β-glucosidase from *Agrobacterium* sp.,[59] whose wild-type enzyme ordinarily catalyzes efficient transglycosylation,[61] recognized α-cellobiosyl fluoride (α-CF) as a monomer and catalyzed formation of $\beta(1\rightarrow4)$ glycosidic linkage. When the catalytic nucleophile Glu at the active site was replaced with a nonnucleophilic residue Ala, the glycosyl-enzyme intermediate could not be formed, and the hydrolysis of the resulting cellooligosaccharide was suppressed.[59] The active-site mutated glycoside hydrolases, which catalyzed the formation of glycosidic linkage but did not hydrolyze the product, were called "glycosynthases". It catalyzed the glycosylation reaction of α-sugar fluorides to produce oligomers up to tetrasaccharides.

A mutated endotype β-glucosidase (E134A) from *Bacillus licheniformis* also showed no hydrolytic activity, yet a new $\beta(1\rightarrow4)$ glycosidic linkage was formed with different glucoside acceptors through a single-step inverting mechanism, when α-laminaribiosyl fluoride was used as donor.[60]

Synthesis of cellulose and cellooligosaccharides catalyzed by a mutated cellulase was carried out by the E197A mutation of recombinant retaining cellulase endoglucanase I (Cel7B) from *Humicola insolens*, using α-CF monomer.[62] Cel7B E197S (E = Glu, S = Ser) was prepared and catalytic constants of both enzymes for the condensation of α-lactosyl fluoride (donor) with *p*-nitrophenyl β-cellobioside (acceptor) were determined by the release rate of the fluoride. Then, the value of k_{cat} of Cel7B E197S was 40 times larger than that of Cel7B E197A.[63]

Further, glycosylation of α-CF or its derivatives to various unsubstituted and modified mono- or disaccharide acceptors was achieved.[62,64] In all cases, the mutated enzyme catalyzed the formation of $\beta(1\rightarrow4)$ glycosidic linkage with regioselective and stereocontrolled manner in good yields. Cel7B mutant recognized branched oligosaccharides containing α-D-xylosyl-$(1\rightarrow6)$-β-D-residues[65−67] and flavonoid[68] as an acceptor, catalyzing the formation of $\beta(1\rightarrow4)$ glycosidic linkage. Interestingly, a triple mutant of Cel7B E197A H209A A211T (H = His, T = Thr) catalyzed the formation of $\beta(1\rightarrow3)$ glycosidic linkage.[69]

Rice (*Oryza sativa*) BGlu1 β-glucosidase (EC 3.2.1.21) is an exotype glycoside hydrolase, which is active on *p*-nitrophenyl (*p*NP)-β-glycoside substrates. The E414G (G = Gly) mutant of rice BGlu1 β-glucosidase catalyzed the formation of $\beta(1\rightarrow4)$ glycosidic linkage from α-D-glucosyl fluoride glycosyl donor and *p*NP-cellobioside glycosyl acceptor, giving rise to cellooligosaccharides as white precipitate in 70−80% yields.[70] The polymer chain length was controlled by the initial feed ratio of glycosyl donor (D) and acceptor (A). Cellooligosaccharides with DPs from 3 to at least 11 were obtained from the polymerization at initial D/A ratio of 5. This seems the first example reporting that exoglycosynthase produced cellooligosaccharide with a longer chain.[70]

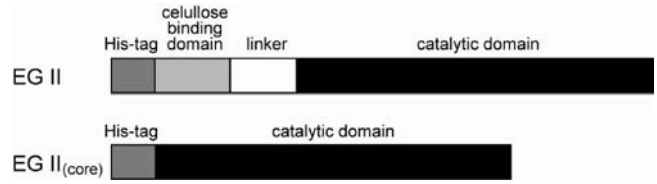


Figure 5. Schematic representation of EG II expressed by *Saccharomyces cerevisiae* and the mutant EG $II_{(core)}$. (Reprinted with permission from ref 41. Copyright 2007 The Japan Academy.)

In the cellulase-catalyzed hydrolysis of crystalline cellulose, cellulose-binding domain (CBD) having an affinity only for crystalline cellulose, helps the enzyme-access to the insoluble substrate. Upon binding of the CBD to crystalline cellulose, the molecular packing of cellulose chains are loosened to accelerate a consecutive cleavage reaction by the catalytic domain.[71]

The second approach of the mutation is using a CBD-deleted mutant cellulase as catalyst. The hydrolysis of the resulting cellulose was considered to be suppressed. A mutant enzyme of cellulase (endoglucanase II, EGII, from *Trichoderma viride*) lacking the CBD was expressed in yeast (Figure 5). The enzyme, $EGII_{(core)}$, catalyzed the polymerization of β-cellobiosyl fluoride (β-CF) to form synthetic cellulose with $\beta(1\rightarrow4)$ glycosidic linkage. As expected, the resulting crystalline product was hardly hydrolyzed by the mutant enzyme.[72] This result indicated that CBD is not important for inducing the polymerization. A new mutant enzyme, $EGII_{(core)2}$, having sequential two active sites of EGII was prepared. $EGII_{(core)2}$ enzyme showed higher polymerization and hydrolysis activities than $EGII_{(core)}$. This was considered mainly because the suitably stabilized conformation with the sequential arrangement.[73]

3.1.1.4. High-Order Self-Assembly of Synthetic Cellulose. There are typically two types of allomorphs of high-ordered molecular structure in cellulose. One is thermodynamically metastable cellulose I, in which cellulose chains are aligned in parallel. The other is thermodynamically stable antiparallel cellulose II. Surprisingly, naturally occurring cellulose forms less stable cellulose I crystalline structure.

Crystalline structures of cellulose synthesized via cellulase-catalyzed polymerization were cellulose II with crude enzyme and cellulose I with purified enzyme. The reason was explained; the polymerization catalyst of cellulase formed a micellar molecular assembly in an acetonitrile/buffer mixed solution. Crude enzyme is believed to contain active sites in much less than 1% for the total enzyme protein. Therefore, active sites of cellulase are separately distributed on the micelle surface. As the result, the elongating cellulose chains formed inter- and intramolecular hydrogen bonds because of the thermodynamically controlled process, giving rise to the cellulose II.[74,75] On the other hand, active sites of the purified cellulase were densely aligned on the surface of the cellulase micellar assembly, and many cellulose chains elongated from these catalytic domains with the same direction. Accordingly, metastable cellulose I could be formed because of the kinetically controlled process.[74,76] This was the first instance of cellulose I formation via a nonbiosynthetic pathway. Such control in high-order molecular assembly during polymerization was not observed before; therefore, a new concept of "choroselective polymerization" was advanced.[77]

A self-assembling process of synthetic cellulose during cellulase-catalyzed polymerization was investigated in detail

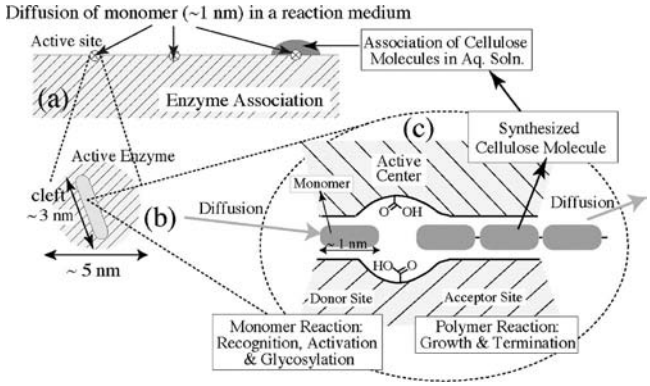


Figure 6. Schematic illustration of cellulose synthesis at a specific reaction field of enzyme associations to be observed by the combined SAS method. (Reprinted with permission from ref 78. Copyright 2007 American Chemical Society.)

at real time and in situ by a combined small-angle scattering (SAS) method, combining small-angle neutron scattering (SANS), small-angle X-ray scattering (SAXS), ultra-SANS, and ultra SAXS methods, together with wide-angle X-ray scattering (WAXS) and field-emission scanning electron microscopy (FE-SEM).[78−80] It was disclosed that cellulase aggregates into associations with characteristic lengths larger than 200 nm in aqueous reaction medium. Further, cellulose molecules created at each active site of enzymes associate themselves around the enzyme associations into cellulose aggregates having surface fractal dimensions D_s, increasing from 2 (smooth surface) to 2.3 (rough surface with fractal structure) with the reaction time progress. The fractal structure formed at the end of the reaction extends over a surprisingly wide length scale ranging from ~30 nm to ~30 μm with 3 orders of magnitude (Figures 6 and 7).[78] This unique self-assembly is caused by an extremely large number of cellulose molecules repeatedly created at the active center of the enzyme. The resulting molecules keep springing out from the narrow catalytic domain of the enzyme toward the reaction medium. Because of the poor solubility of cellulose in the reaction medium, synthetic cellulose associates into aggregates.

3.1.2. Amylose

3.1.2.1. Amylase-Catalyzed Polycondensation. Amylose is a polymer of glucose linked through α(1→4) glycoside bonds. By polycondensation of α-D-maltosyl fluoride using α-amylase as catalyst in a methanol−phosphate buffer (pH 7.0), 2:1 (v/v) mixed solution, amylose oligomers up to heptasaccharide were synthesized (Scheme 7).[81] From the high-performance liquid chromatography (HPLC) analysis, formation of odd-numbered maltooligosaccharides was also observed, suggesting that enzymatic hydrolysis or transglycosylation of the products occurred in parallel with the polymerization. Steric hindrance caused from the helical structure of amylose might become a barrier for producing longer molecules.

3.1.2.2. Phosphorylase-Catalyzed Polycondensation. Phosphorylase (EC 2.4.1.1) is an exotype enzyme, which catalyzes in vivo phosphorolysis at the nonreducing end of the glycosidic linkage. The enzyme can be utilized for glycosylation reaction because the substrate of a glycosyl-phosphate is activated and possesses the relatively low bond energy, compared to that of a glycosyl-nucleotide. Phosphorylase-catalyzed reactions in vitro progress in a reversible

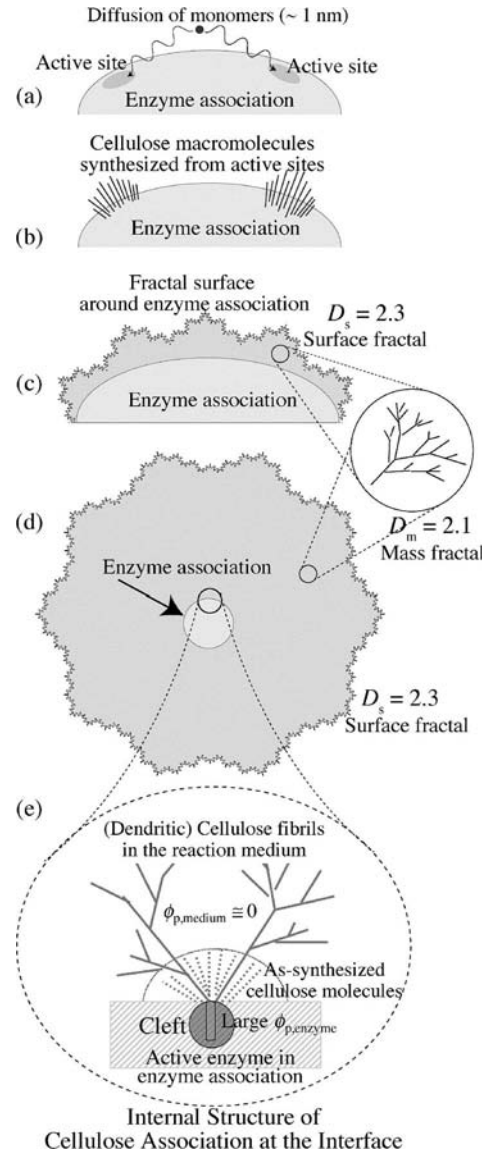
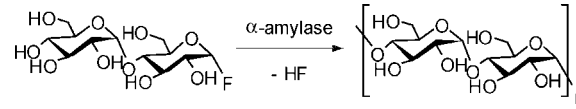


Figure 7. Schematic models of self-assembling process of synthetic cellulose on the surface of enzyme associations. (Reprinted with permission from ref 78. Copyright 2007 American Chemical Society.)

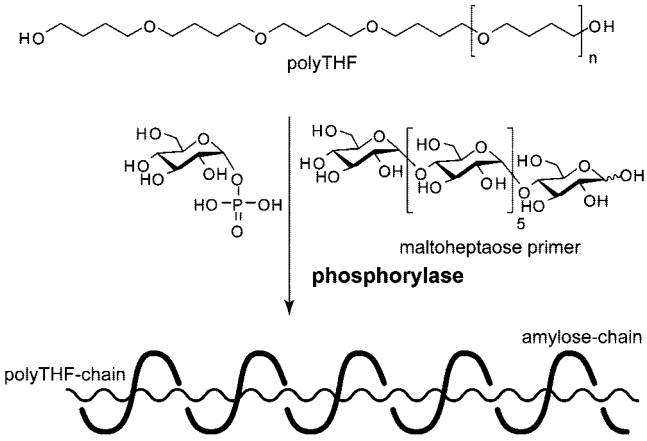
Scheme 7



manner; indeed, it catalyzes both reactions, phosphorolysis and formation of glycosidic linkage.[82] In vitro synthesis of polysaccharides was performed via phosphorylase-catalyzed polycondensation.[83−85]

Amylose was synthesized in vitro by the phosphorylase-catalyzed polymerization of α-D-glucose 1-phosphate (Glc-1-P) monomer, which was initiated from a primer of maltoheptaose. When the polycondensation was carried out in the presence of polyTHF as a hydrophobic polymer, the polymerization proceeded with the formation of the amylose-polyTHF inclusion complex (Scheme 8). From the ^{1}H NMR and GPC measurements, a DP value of the resulting amylose chain was confirmed to be 75−90, which corresponded to 99−119 Å of molecular length in helical form. From the length of polyTHF of 230 Å, one THF molecular unit is

Scheme 8



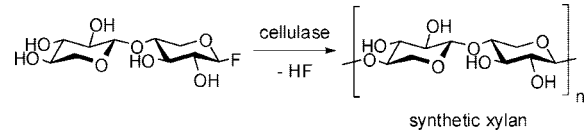
included by two amylose molecular units. This inclusion complex could not be formed from the reaction mixture of amylose and polyTHF with the same polymer length.[86] Formation of amylose-polymer inclusion complexes with various axle molecules was observed using polyethers, polyesters,[87−89] poly(ester-ether),[88] and polycarbonate[90] having appropriate methylene chain length. The hydrophobicity of the guest polymers is an important key-point for the formation of the inclusion complexes, and no-inclusion complex was obtained from hydrophilic polymers such as poly(ethylene glycol).[91] When phosphorylase-ctalyzed polymerization of Glc-1-P was performed in a mixture of polyTHF and poly(oxetane), the product amylose preferentially formed a polyTHF inclusion complex because of the better hydrophobicity.[92] This unique polymerization to produce amylose-polymer inclusion complexes was termed as "vine-twining polymerization".[91,93,94]

When maltooctaose and maltooctaose-phosphate were used for the phosphorylase-catalyzed reaction, polymer length of the resulting polysaccharides was regulated by that of a linear polymeric guest molecule. Molecular weights of the resulting amylose chains were coincident with calculated molecular weight value based on the number of maltooctaose molecules stacked along the molecular length of the guest.[95] The phosphorylase-catalyzed reaction was adapted to various polymerizations. For example, syntheses of amylose grafted chitin and chitosan were reported.[96,97]

Deinococcus geothermalis glycogen branching enzyme (DgGBE, EC 2.4.1.18) is known to catalyze the redistribution of short α-glucans via inter- and intramolecular chain transfer from α(1→4) positions to α(1→6) positions. DgGBE catalyzed the branch formation until the average degree of branching was reached to 11%. By the combinational usage of DgGBE with potato phosphorylase, highly branched amylose could be synthesized from glucose-1-phosphate.[98]

3.1.2.3. Glycosyltransferase Catalyst. Amylosucrase (EC 2.4.1.4) belongs to a glycosyltransferase (GTase), which catalyzes the transfer of glucose moiety to an acceptor molecule. Recombinant amylosucrase from *Neisseria polysaccharea* was used to synthesize amylose in vitro from sucrose monomer without using a primer. The product amylose possessed the degree of polymerization (DP) of 58−35. The morphology and structure of the resulting amylose depended on the initial concentration of sucrose.[99] When glycogen particles were used as primer, amylose-based dendritic nanoparticles were obtained from the enzyme-catalyzed reaction.[100]

Scheme 9



The GTase from *Bacillus macerans* catalyzed a cyclization condensation of 6′-*O*-methyl-α-maltosyl fluoride to afford the tri-*O*-methyl-α-cyclodextrin in 42% yields.[101] The cyclodextrin GTase (CGTase) from *Bacillus circulans* showed the catalysis of the condensation of S-linked α-maltosyl fluorides to give both cyclic and linear oligosaccharides, whereas the C-linked α-maltosyl fluorides produced only linear oligosaccharides.[102]

3.1.3. Xylan

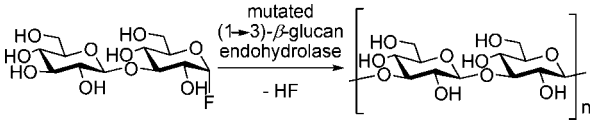
3.1.3.1. Cellulase-Catalyzed Polycondensation. Xylan, one of the most important components of hemicelulose in plant cell walls, is a polysaccharide of xylose with a *β*(1→4) glycosidic linkage. On the basis of the TSAS concept, *β*-xylobiosyl fluoride was designed as monomer for glycosidase-catalyzed polymerization. In a mixed solution of acetonitrile and acetate buffer (pH 5.0), cellulase from *Trichoderma viride*, a commercial crude enzyme known to show xylanase activity, catalyzed the polycondensation of the monomer (Scheme 9).[103,104] The resulting water-insoluble white precipitate was confirmed to be a synthetic xylan having *β*(1→4) glycosidic linkage. The carboxymethylated product showed an average molecular weight of 6.7×10^3, which corresponded to a DP value of 23.

A trisaccharide fluoride monomer was treated with endo-1,4-*β*-glucanase (from *Trichoderma viride*) in acetonitrile/buffer to induce a polycondensation reaction, and xyloglucan oligomers were produced.[105,106]

3.1.3.2. Mutant Xylanase Catalyst. Glycoside hydrolase (GH) family 10 xylanase (EC 3.2.1.32) is an endotype glycoside hydrolase of xylan with retaining hydrolysis manner. The point mutation of catalytic nucleophilic Glu (E) residue to Gly (G) and Ser (S) was conducted using xylanase from *Cellulomonas fumi* (CFXcd), and catalytic behaviors of the mutated two enzymes (CFXcd-E235G and CFXcd-E235S), using an α-xylobiosyl fluoride monomer, were investigated.[107] Only CFXcd-E235G showed glycosynthase activity; however, α-xylobiosyl fluoride was a poor acceptor for the enzyme. When *p*-nitorophenyl *β*-xylobioside or benzylthio *β*-xylobioside was used as glycosyl acceptor, the mutant enzyme catalyzed the formation of *β*(1→4) glycosidic linkage using α-xylobiosyl fluoride as a glycosyl donor. The degree of polymerization of the products was ranged from 4 to 12.

Mutated xylanases from different five origins were prepared,[108,109] whose catalytic nucleophilic amino acid residue was substituted in the same way with CFXcd. These mutated xylanases from *Thermotoga maritime* XylB (TM), *Clostridium stercorarium* XynB (CS), *Bacillus halodurans* XynA (BH), *Cellulomonas fimi* Cex (CF), and *Geobacillus stearothermophilus* XT6 (XT6) were abbreviated to TM-E259G, CS-E293G, BH-E301G, CF-E233G, and XT6B2-E265G, respectively. Former four enzymes catalyzed the homopolymerization of α-xylobiosyl fluoride and gave white precipitates. Structure of the white precipitates was confirmed to be synthetic xylan, and yields catalyzed by TM-E259G, CS-E293G, BH-E301G, and CF-E233G were 32%, 69%, 29%, and 42%, respectively.[108]

Scheme 10



Mutated enzyme of GH family 52 β-xylosidase (EC 3.2.1.37) from *Geobacillus stearothermopilus* (XynB2-E335G) catalyzed the dimerization of monosaccharide α-xylopyranosyl fluoride, giving rise to α-xylobiosyl fluoride. No polymerized products were obtained from monosaccharide monomer of α-xylopyranosyl fluoride by the catalysis of XT6B2-E265G. However, starting from α-xylopyranosyl fluoride, white precipitate was obtained with two enzymes XynB2-E335G and XT6B2-E265G. The resulting white precipitate was synthetic xylan and its DP was ranged from 6 to over 100.[109] It is considered that after the production of α-xylobiosyl fluoride via XynB2-E335G-catalyzed dimerization, the resulting disaccharide fluoride was recognized by XT6B2-E265G and polymerized. This seems to be a facile method for xylan synthesis.

3.1.3.3. Transglycosylase-Catalyzed Polycondensation. Xylogucan endotransglycosylase (XET) (EC 2.4.1.207) is a transglycosidase, which is involved in the rearrangement of xyloglucans by endolytic cleavage and relegation. XET is structurally and mechanically related to microbial glycosyl hydrolases and grouped into glycoside hydrolase (GH) family 16. The E85A mutated XET from *Populus tremula* and *Populus tremuloides* (*Ptt*XET16-34) catalyzed homo- and heterocondensations of α-xylogluco-oligosaccharyl fluoride donors, giving rise to xyloglucans with regular side chain substitution patterns. From the reaction mixture, oligomers up to $n = 10$ were obtained.[110]

3.1.4. (1→3)-β-D-Glucans

Polysaccharides such as curdlan, laminarin, sizofiran, etc., are β(1→3) linked D-glucose polysaccharides and are generically called (1→3)-β-D-glucans. It is known that there are some β(1→6), β(1→4), or both side chains branching off from the β(1→3) linked main chain of natural (1→3)-β-D-glucans. They are widely distributed in nature, such as those from plants, fungi, and bacteria, and have various names according to their origins. For example, those derived from seaweed and high plants are named as laminaran and callose, respectively. (1→3)-β-D-Glucans have attracted much attention because of their high immunostimulatory and anticancer activities.[111]

(1→3)-β-D-Glucan endohydrolase (EC 3.2.1.39) is an enzyme, which hydrolyzes β(1→3) glycosidic linkage of (1→3)-β-D-glucans. Polymerization of a β-laminaribiosyl fluoride monomer was carried out, using a wild-type (1→3)-β-D-glucan endohydrolase isoenzyme GII as a catalyst; however, the overall yields of the products were low.[112] Then, three types of mutated (1→3)-β-D-glucan endohydrolases were prepared, whose catalytic nucleophile Glu (E) residue at 231 was replaced with Gly (G), Ser (S), or Ala (A) by site-directed mutagenesis. All mutated enzymes catalyzed the transglycosylation of α-laminaribiosyl fluoride monomer in a mixed solvent of acetate buffer (pH 5.0) and CH_3CN (Scheme 10). Mutated E231G enzyme showed higher catalytic efficiency than the other two enzymes, and DP values of the resulting polysaccharides ranged from 28 to 44. The mutated E231G enzyme also catalyzed the polymerization of 3-thio-α-laminaribiosyl fluoride monomer, giving rise to the corresponding polysaccharide with DP values of 6−18. Electron and X-ray diffraction patterns indicated that the resulting polysaccharide chains took parallel and triple helical conformation.[112]

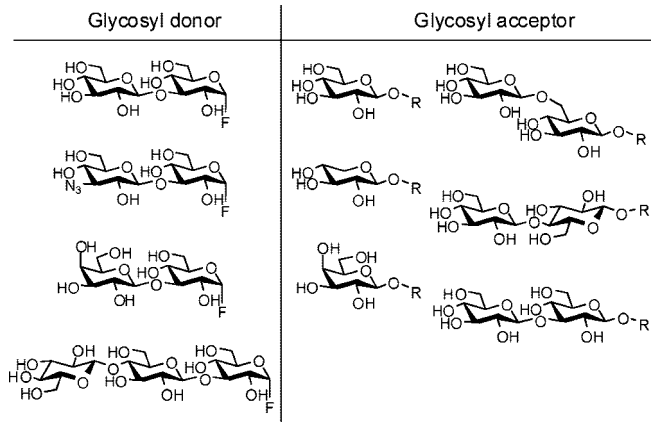
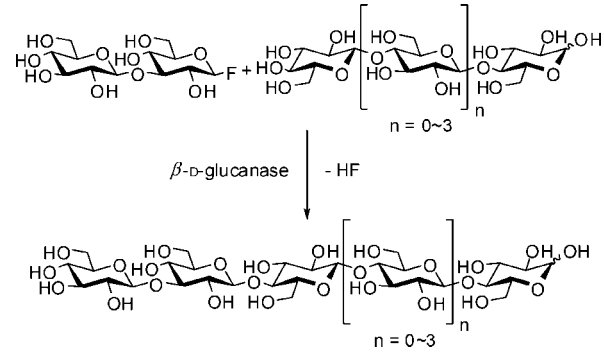


Figure 8. Formation of a β(1→3) glycosidic linkage via condensation of α-laminaribiosyl fluoride and its derivatives as donors with a variety of mono- and disaccharide acceptors catalyzed by the mutated E231G (1→3)-β-D-glucan endohydrolase.

Scheme 11

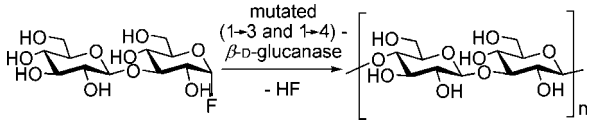


Enzymatic synthesis of a β(1→3) glycosidic linkage via condensation of α-laminaribiosyl fluoride and its derivatives with a variety of mono- and disaccharide acceptors was investigated using the mutated E231G enzyme as catalyst. The enzyme catalyzed various glycosylation reactions (Figure 8).[113]

3.1.5. Other Polysaccharides

(1→3, 1→4)-β-D-Glucan is one of the major cell wall components of the higher plants other than cellulose. In this polysaccharide, the content of β(1→3) glycosidic linkages usually does not exceed 25−30%. (1→3, 1→4)-β-D-Glucan endohydrolase (EC 3.2.1.73) is an enzyme, which catalyzes bond cleavage of β(1→4) glycosidic linkages on 3-O-substituted glycopyranosyl residues. The glucanase obeys a retaining hydrolysis mechanism, which involves a double displacement reaction assisted by general acid/base catalysis. Glycosylation of β-laminaribiosyl fluoride with excess amounts of cellooligosaccharide glycosyl acceptors was examined using a (1→3, 1→4)-β-D-glucananase from *Bacillus licheniformis* (Scheme 11).[114] In a mixed solution of sodium maleate buffer (pH 7.0) and acetonitrile at 40 °C, the enzyme catalyzed the formation of a β(1→4) glycosidic linkage in yields of 15−45%. Further experiments indicated that the E134A mutant of (1→3, 1→4)-β-D-glucananase from *Bacillus licheniformis* catalyzed the formation of β-glycosidic

Scheme 12



linkages with the 4−OH group of the glucan acceptor in an equatorial configuration.[115,116] In vitro synthesis of (1→3, 1→4)-β-D-glucans was performed via polycondesation catalyzed by the mutated (1→3, 1→4)-β-D-glucananase from *Bacillus licheniformis*.[117] The catalytic nucleophilic residue of Glu134 (E) was point-mutated to Ala (A). The resulting E134A mutant enzyme induced the polymerization of α-laminaribiosyl fluoride monomer, giving rise to a polysaccharide with an alternatingly aligned β(1→3)-Glc and β(1→4)-Glc structure (Scheme 12). The DP values of the polysaccharide ranged from 6 to 12. The polysaccharide formed a spherical assembly and its crystalline structure was confirmed by X-ray and electron diffraction analyses.

The synthesis of poly-*N*-acetyllactosamine was performed by the catalysis of endo-β-galactosidase (EC 3.2.1.103) from *Escherichia freundii*. The enzyme induced regioselective transfer of di- and tetrasaccharide units onto 4-OH position of GlcNAc residue at the nonreducing terminal end of acceptor substrates.[118,119] Reaction efficiency of the polysaccharide production was enhanced by the addition of bovine serum albumin (BSA) and by a low-temperature reaction condition.[119] The β-D-mannopyranoside linkage is one of the most difficult glycosidic bonds to form via conventional organic chemistry method. E519S mutant of *Cellulomonas fimi* β-mannosidase (Man2a) catalyzed the formation of the β(1→3) D-mannopyranoside linkage and gave oligosaccharides from the reaction of α-D-mannosyl fluoride.[120]

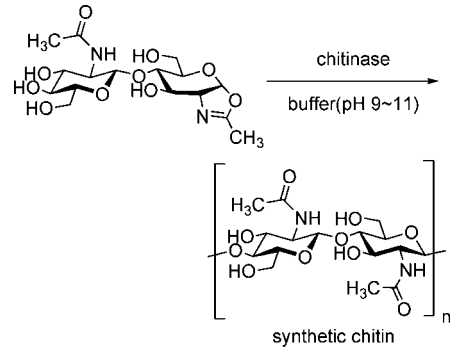
3.2. Natural Polysaccharides and Their Derivatives via Ring-Opening Polyaddition

3.2.1. Chitin and Its Derivatives via Chitinase-Catalyzed Ring-Opening Polyaddition

3.2.1.1. Chitin. Chitin is a β(1→4)-linked *N*-acety-D-glucosamine (GlcNAc) polysaccharide, which is synthesized in vivo by the catalysis of chitin synthase (EC 2.4.1.16) with UDP-GlcNAc as a substrate.[121] Chitosan is an *N*-deacetylated product of chitin. They are among the most abundant and widely distributed polysaccharides in the animal field as a structural material and show excellent characteristics of biodegradability, biocompatibility, and in particular, low immunogenicity. Therefore, they are frequently employed as a starting material in medicine, pharmaceuticals, cosmetics, and food supplements.[122] The first in vitro synthesis of chitin was accomplished in 1995 via ring-opening polyaddition of a chitobiose oxazoline derivative monomer (a disaccharide) catalyzed by chitinase (EC 3.2.1.14), a glycoside hydrolase of chitin, and published in 1996 (Scheme 13).[36,123−125] The reaction is of ring-opening polyaddition mode, and it was promoted by family 18 chitinase under even weak alkaline conditions (pH 9.0−11.0), where the hydrolysis of the product chitin was much suppressed. The DP value of "synthetic chitin" was evaluated as 10−20 depending on the reaction conditions employed.[41]

As to the mechanism of the chitinase catalysis, it is accepted that two carboxylic acid residues are involved for the catalysis (Figure 9). In the hydrolysis of chitin, oxygen atom of glycosidic linkage is protonated by one of the

Scheme 13



carboxylic residues immediately after the recognition of chitin at the catalytic domain (stage a). Carbonyl oxygen atom of C-2 acetoamide group is attacked by the neighboring anomeric carbon from the α-side to form an oxazolinium stabilized by another carboxylic residue, and the C-1−O bond cleavage is completed (stage b). Therefore, the reaction is of "substrate assisted mechanism".[126] Ring-opening of this oxazolinium ring occurs by nuclephilic attack of water molecule from β-side, giving rise to the hydrolysate in a retaining manner (stage c). It is considered that the oxazolinium ion formation is a transition-state (or intermediate) in the hydrolysis.

In the polymerization, a chitobiose oxazoline derivative was developed as a transition-state analogue substrate (TSAS) monomer for chitinase catalyst. First, the monomer is recognized and at the same time the nitrogen atom is protonated by the carboxylic acid residue at the donor site of chitinase, forming the corresponding oxazolinium ion (stage b′). This is because the monomer has already an oxazoline structure. Instead of the water molecule, C-4 hydroxy group of sugar unit located at the acceptor site attacks nucleophilically the anomeric carbon from the β-side to open the ring, providing a new glycosidic linkage with β-configuration (step c′). Thus, the monomer acted as a glycosyl donor, as well as a glycosyl acceptor, and this glycosidic linkage formation reaction is repeated during the polymerization. The most important point for the chitinase-catalyzed polymerization is the structure resemblance of the transition-state (or intermediate) involved in both reactions at stage b and stage b′. From these considerations a new concept of TSAS was proposed,[36] and then a mechanism involving an oxazolinium ion intermediate in the chitin hydrolysis by family 18 chitinase was reported.[126] During the polymerization, 2-methyl-oxazoline ring of the monomer played a role of forming *N*-acetyl group by opening the ring; the ring can be taken as a masked *N*-acetyl group.

When the oxazoline of GlcNAc (a monosaccharide) was used as monomer, chitooligosaccharides were produced. These reactions were the first examples, demonstrating that a sugar oxazoline can be utilized as a glycosyl donor for an enzymatic glycosylation.[127,128]

Further, to take advantage of the high reaction specificity of enzymes, stepwise elongation of GlcNAc unit was performed via combined use of chitinase and β-galactosidase. Chitinase recognized the 4-OH group of GlcNAc as an acceptor. Therefore, by the catalysis of the enzyme, no self-polyaddional products were produced from *N*-acetyllactosamine (LacNAc) oxazoline derivative, which is a disaccharide of galactose (Gal)β(1→4)GlcNAc. Chitinase catalyzed the glycosylation of LacNAc oxazoline derivative to the

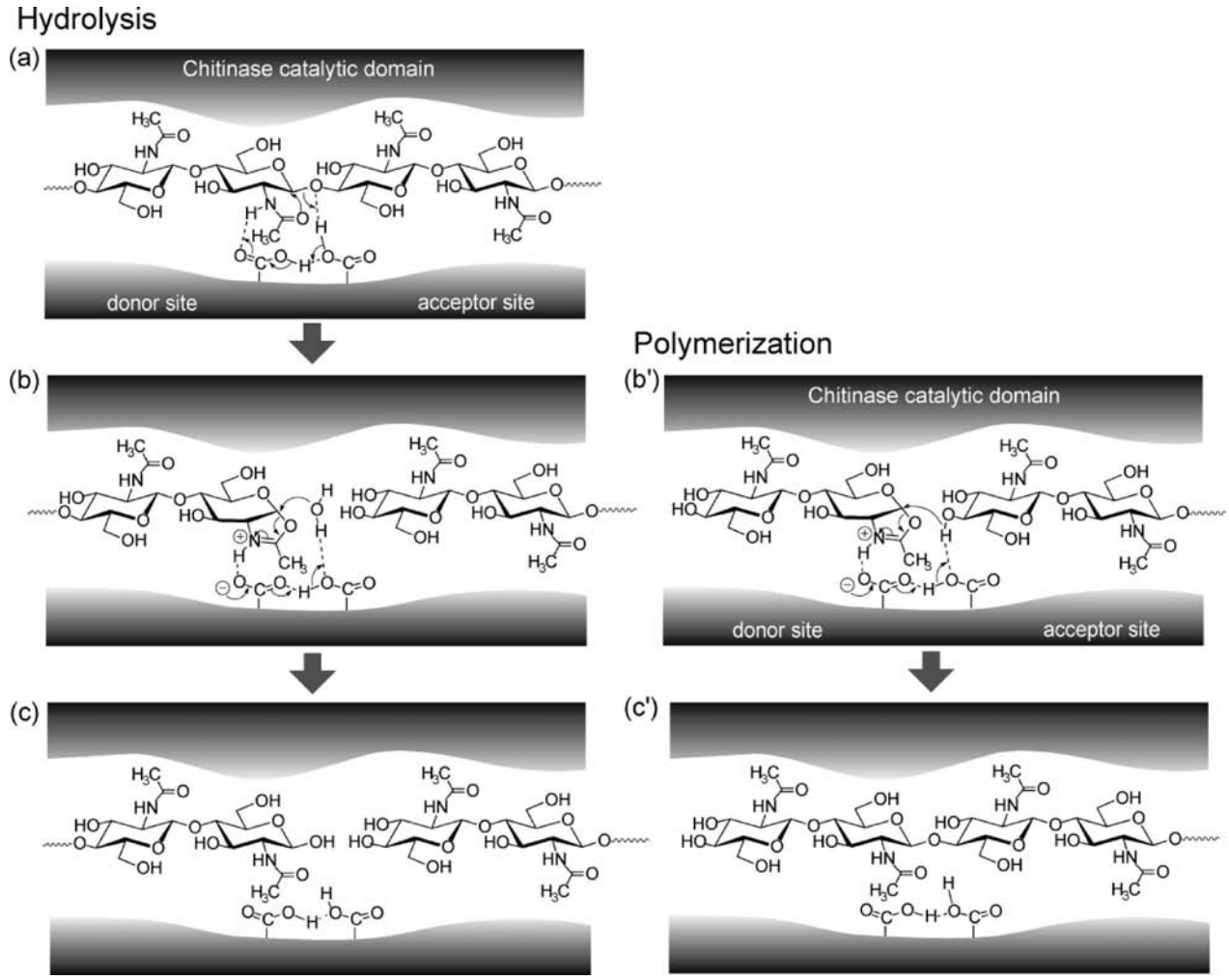
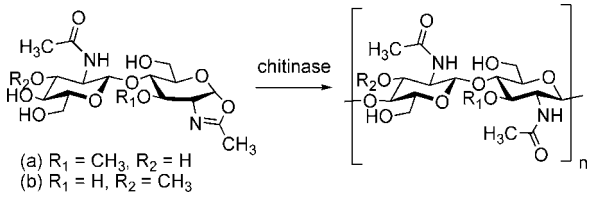
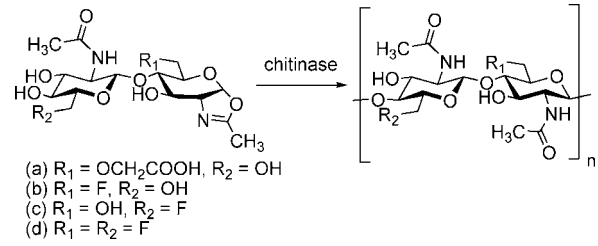


Figure 9. Postulated reaction mechanisms of chitinase catalysis for hydrolysis of chitin (left) and for ring-opening polyaddition of the chitobiose oxazoline monomer to synthetic chitin (right). (Reprinted with permission from ref 41. Copyright 2007 The Japan Academy.)

Scheme 14



Scheme 15



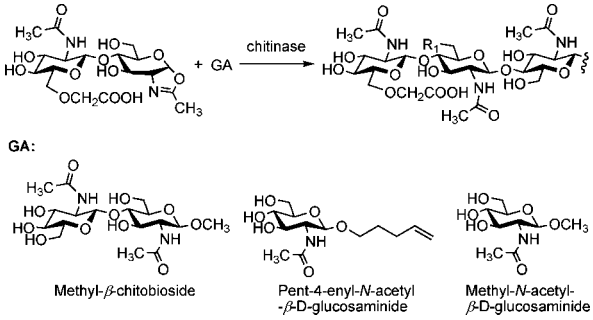
4-OH group of GlcNAc acceptor. After the glycosylation, the Gal unit located at nonreducing end of the product was removed via β-galactosidase-catalyzed Galβ(1→4)GlcNAc glycosidic bond cleavage. Repetitions of these sequential manipulations enabled to synthesize chitooligosaccharide with desired chain lengths.[129,130]

3.2.1.2. Chitin Derivatives. In the chitinase-catalyzed synthesis of chitin, the oxazoline structure at reducing end and C-4 hydroxy group at nonreducing end are essential for the exclusive formation of β(1→4) glycosidic linkage. A possibility as the substrate was examined by modifying the TSAS monomer of C-3 hydroxy group. 3-*O*-Methyl, and 3′-*O*-methyl-chitobiose oxazoline derivatives were designed as new TSAS monomers (Scheme 14).[131] Both monomers produced only chitooligosaccharides in rather low yields.

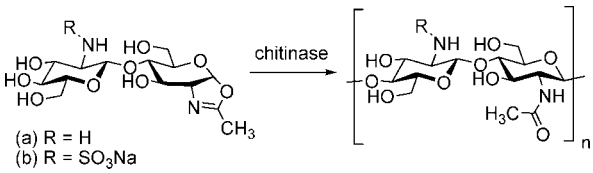
To examine the substituent effects, 6-*O*- or 6′-*O*-carboxylmethylated (CM) chitobiose oxazoline derivatives were designed and polymerized by chitinase, indicating that both monomers were recognized by donor site of chitinase. However, resulting product was dimer (tetrasaccharide) from the reaction of 6′-*O*-CM chitobiose oxazoline derivative (Scheme 15a).[132,133] 6-*O*-CM derivative showed no glycosylation reaction probably because of the steric hindrance. This prediction was supported from the result that only 6′-*O*-CM chitobiose oxazoline derivative gave corresponding tri- or tetrasaccharide in good yields by chitinase-catalyzed glycosylation with methyl-β-chitobioside, pent-4-enyl-*N*-acetyl-β-D-glucosaminide, and methyl-*N*-acetyl-β-D-glucosaminide (Scheme 16).[132]

Chitobiose oxazoline derivatives having C-6 fluorine substituent were prepared because of the similar size of the fluorine atom to oxygen atom, and hence similar steric factor between OH and F as subtituent at C-6 (Scheme 15b−d).[134,135] Under the weak alkaline conditions, reactions of these three monomers were induced by chitinase, giving rising to the

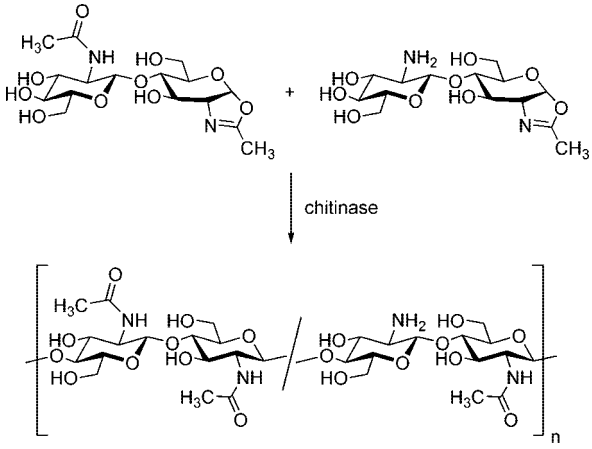
Scheme 16



Scheme 17



Scheme 18



white precipitates of structurally well-defined fluorinated-chitins. The average molecular weights of F-chitins were 1400−1700.

An *N*-acetylchitobiose oxazoline derivative, whose non-reducing end of the *N*-acetyl group was deacetylated, was polymerized by chitinase catalyst to produce the corresponding polysaccharide having alternatingly aligned *N*-deacetylated group. Chitosan is an *N*-deacetylated product of chitin, which has a $\beta(1\rightarrow4)$ linked D-glucosamine (GlcN) structure. Therefore, the resulting polysaccharide was named "chitin−chitosan hybrid polysaccharide" (Scheme 17a).[136] During the polymerization, the reaction mixture kept homogeneous. M_n value of chitin−chitosan hybrid polysaccharide was 1670. Chitinase from *Serratia marcescens* also effectively catalyzed the polymerization and gave corresponding polysaccharide in a yield of 75% with $M_n = 2020$.

By copolymerization of *N*-acetylchitobiose oxazoline monomer with *N*,*N*′-diacetylchitobiose oxazoline monomer, tailor-made synthesis of a chitin derivative with controlled deacetylated extent ranging from 0% to 50% was performed (Scheme 18).[137] Family 18 chitinase from *Bacillus* sp. showed close polymerization activity for both comonomers, and the deacetylated extent of the copolymer was controlled by the initial comonomer feed ratio.

A TSAS monomer bearing a bulky *N*-sulfonate group at the C-2′ position was designed (Scheme 17b).[138] Chitinase-catalyzed polymerization proceeded homogenously, due to a good solubility of the resulting polysaccharide. By selecting appropriate chitinase enzymes, molecular weight was controlled to some extent. For example, chitinase from *Aeromonas hydrophila* and *Serratia marcescens* provided a dimer (tetrasaccharide) and a polysaccharide of $M_n = 4180$, respectively.

The catalytic domain of family 18 chitinase consists of a $(\beta/\alpha)_8$ barrel with a deep substrate-binding cleft. From the X-ray diffraction measurement of crystalline family 18 chitinase, it was revealed that C-2 acetamido groups located at subsites −1 and +2 face to deep inside of the cleft and interact with aromatic residue, but those at subsites −2 and +1 face to opposite sides of the cleft.[139] Therefore, chitobiose oxazoline derivatives modified at C-2′ *N*-acetamido group was polymerized as shown in Scheme 17b. On the other hand, location of C-3 and C-6 hydroxy groups looks relatively busy.

3.2.1.3. Mutant Chitinase Catalyst. To suppress the chitinase-catalyzed hydrolysis of monomer or resulting polysaccharide, mutation of chitinase was performed. It was found before that a DXE (D = Asp, X = any amino acid, and E = Glu) motif is conserved at the catalytic domain of chitinase enzyme.[140,141] In fact, wild-type chitinase A1 from *Bacillus circulans* WL-12 has D202 and E204 residues; D202 serves as a stabilizer for the oxazolinium ion during the hydrolysis. In the polymerization reaction, this D202 residue would be responsible for the N-protonation of the oxazoline ring and helping a nucleophilic attack of acceptor C-4 hydroxy group to anomeric carbon (see, Figure 9). E204 would protonate oxygen atom of the glycosyl linkage to be cleaved. Then, a mutant chitinase E204Q, where COOH group of Glu was replaced with $CONH_2$ group of Gln to lack the protonation ability, was prepared. As expected, the mutated enzyme (E204Q) showed catalytic activity of the oligomerization of the chitobiose oxazoline monomer but lacked the hydrolysis activity of the resulting oligosaccharides.[142]

3.2.1.4. High-Order Self-Assembly of Synthetic Chitin. The self-assembly process of crystal chitin during the chitinase-catalyzed polymerization was investigated using phase-contrast and polarization microscopy in combination with SEM and TEM.[143] Under the reaction conditions of pH 10.5 and 30 °C, it took 25 h for the complete consumption of the monomer. During the first 30 min, a small number of rectangular platelike solids were observed, whose width, height, and length were 25 nm, 10 nm, and 50 nm−1 μm, respectively. The electron microdiffraction patterns of the plates agreed well with that of thermodynamically stable form of chitin crystal of α-chitin. Therefore, it was a single crystalline plate of α-chitin, in which polysaccharide chains formed an antiparallel structure via intra- and intermolecular hydrogen bonding. The crystal plate grew and stacked each other as time elapsed and shaped into ribbons, followed by formation of bundle-like assemblies in 3 h observed by TEM, leading to synthetic chitin spherulites (Figure 10).

3.2.2. Glycosaminoglycans

Glycosaminoglycans (GAGs) are polysaccharides, which are usually linked to various proteins to form proteoglycans (with a sole exception of hyaluronan). Proteoglycans, collagens, and fibronectins fill the interstitial space between living cells, named extracellular matrices (ECMs), and act as a compression buffer against the stress placed in the

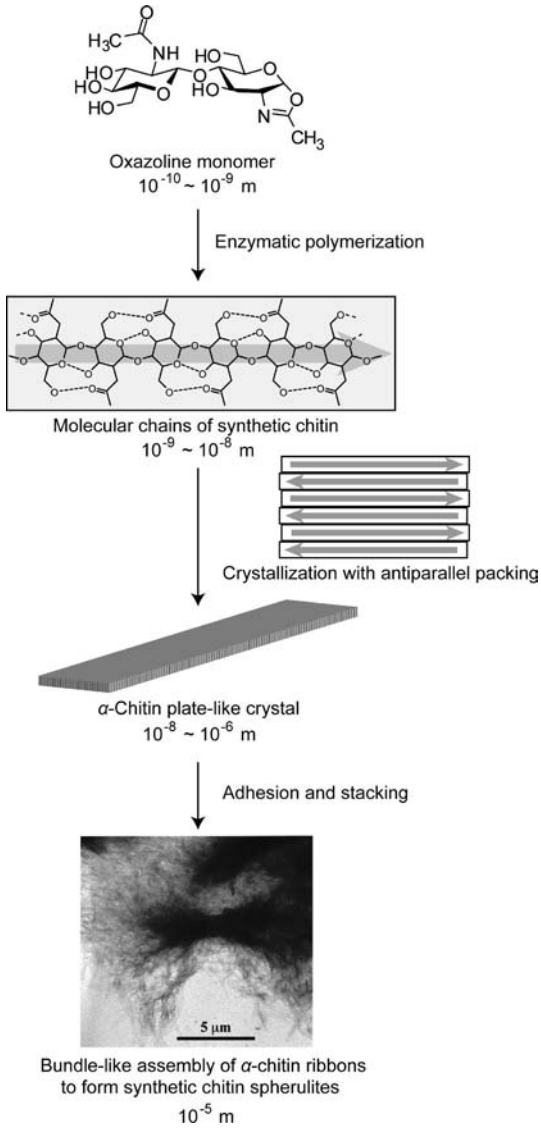


Figure 10. Hierarchy structures of synthetic chitin in vitro formed via enzymatic polymerization. (TEM image: Reprinted with permission from ref 143. Copyright 2000 American Chemical Society.)

ECMs.[144] Therefore, GAGs are considered to play important roles in living organisms, and are frequently used as therapeutic materials and food supplements. GAGs are heteropolysaccharides consisted from a hexosamine and an uronic acid (Figure 11).[145] Some GAGs have various sulfonation patterns. Therefore, to develop a new synthetic method of GAGs with well-defined structure is one of the most important strategies for the elucidation of GAG functions at a molecular level. The hexosamine unit of GAGs is *N*-acetyl-D-glucosamine (GlcNAc) or *N*-acetyl-D-galactosamine (GalNAc). Some glycoside hydrolases would involve an oxazolinium intermediate during the hydrolysis. Therefore, from an extension of family 18 chitinase, disaccharide monomers having an oxazoline structure were newly designed based on the TSAS concept for in vitro synthesis of GAGs.

3.2.2.1. Hyaluronan and Its Derivatives. Hyaluronan (hyaluronic acid, HA) is a nonsulfated GAG and one of the major component of ECMs. HA plays important functions in vivo, for example, tissue proliferation, regeneration, wound healing and so forth. HA has a disaccharide repeated structure of D-glucronic acid (GlcA)-β(1→3)-*N*-acetyl-D-glucosamine (GlcNAc) connecting through β(1→4) glycosidic linkage.

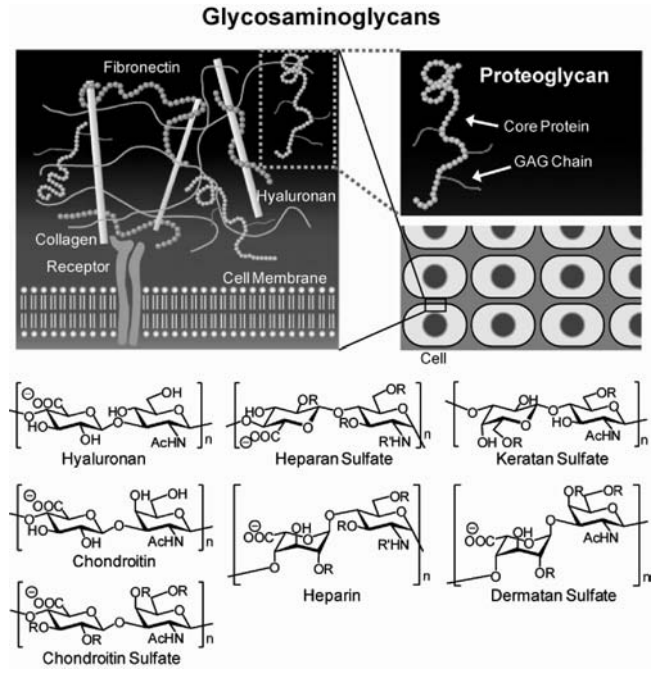
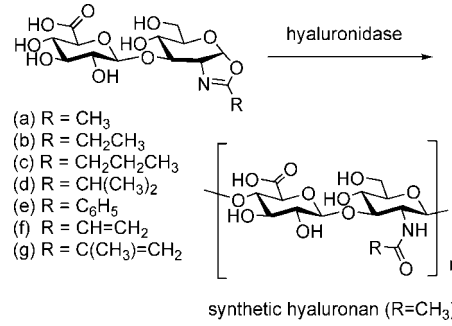


Figure 11. Glycosaminoglycan (GAG) chains are linked to core proteins, which form proteoglycans. Chemical structures of typical seven GAGs are given. (Reprinted with permission from ref 41. Copyright 2007 The Japan Academy.)

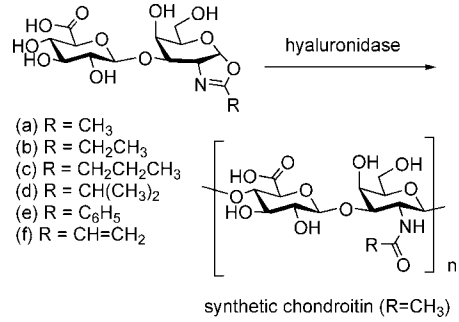
Scheme 19



Family 56 hyaluronidase (HAase, EC 3.2.1.35) is an endotype glycoside hydrolase, which hydrolyzes β(1→4) glycosidic linkage between GlcNAc and GlcA. With an extension of the chitin synthesis involving a GlcNAc donor site, a novel GlcAβ(1→3)GlcNAc oxazoline derivative was designed as a TSAS monomer and its HAase-catalyzed polymerization was examined using ovine testicular HAase (OTH) and bovine testicular HAase (BTH). At pH 7.1−9.0, both enzymes induced the polymerization of the monomer, giving rise to HA with an M_n value of 1.74×10^4 in 52% yields by OTH catalyst and HA with M_n value of 1.35×10^4 in 39% yields by BTH catalyst. Regio- and stereochemistry of the resulting HA was perfectly controlled (synthetic hyaluronan) (Scheme 19).[37,146] The hydrolysis mechanism of HAase is considered to involve a double displacement mechanism, which is similar to that of family 18 chitinase.[147,148] Synthetic HA seems to be an example polymer having one of the most complicated structures ever prepared in vitro.

Cross-linked HA samples are utilized as artificial ECMs. These samples have been prepared via chemical modification of natural HA; however, to control the chemical modification is difficult and unexpected side reactions containing bond cleavage of the HA chain cause the molecular weight reduction. For extension of the HA synthesis, HAase-

Scheme 20



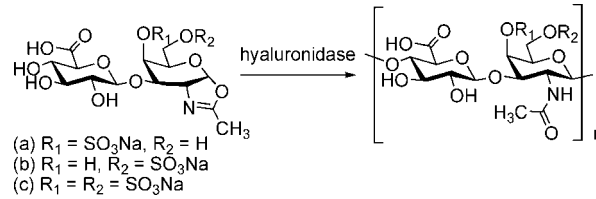
catalyzed polymerization to HA derivatives bearing *N*-propinoyl, *N*-butyryl, *N*-isobutyryl, *N*-benzoyl, *N*-acryloyl, and *N*-methacryloyl functional groups in place of the *N*-acetyl group was achieved (Scheme 19).[149] The reactivity of the monomers was in a following order: 2-methyl (natural type) > 2-ethyl ≈ 2-vinyl > 2-*n*-propyl ≫ 2-isopropyl > 2-isopropenyl ≈ 2-phenyl.[149] A HA derivative having *N*-acryloyl group has a reactive vinyl group, and hence, functions as maromonomer, leading eventually to telechelics and graft copolymers. Further, HAase-catalyzed copolymerization between different two HA oxazoline monomers produced HA derivatives, where the copolymer composition having different *N*-acyl groups was controlled by varying the monomer feed ratio.[150]

3.2.2.2. Chondroitin and Chondroitin 4-Sulfate. Chondroitin (Ch) is a sulfated GAG composed of alternatingly aligned *N*-acetyl-D-galactosamine (GalNAc) and D-glucronic acid (GlcA). Chondroitin sulfate (ChS) in nature is known to contain four kinds according to the site of sulfation. Ch sulfated at C-4 of GalNAc, C-6 of GalNAc, C-2 of GlcA, C-6 of GalNAc, and C-4 and C-6 of GalNAc are named ChS-A, ChS-C, ChS-D, and ChS-E, respectively. A number of papers have been published, describing the functions of ChS at a molecular level and the various sulfation pattern confers specific biological activities.

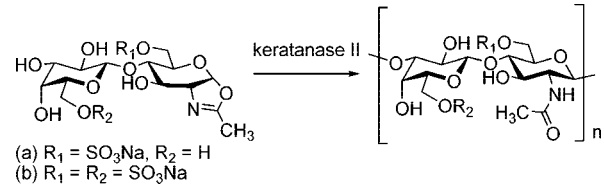
The structural difference between HA and Ch main chains is the difference in stereochemistry of C-4 in hexosamine unit. GalNAcβ(1→4)GlcA glycosidic linkage in Ch and ChS is known to be hydrolyzed by family 56 HAase. Therefore, a new oxazoline derivative (2-methyl), as well as 2-ethyl, 2-*n*-propyl, 2-isopropyl, 2-phenyl, and 2-vinyl oxazoline derivatives, was designed as new TSAS monomers for hyaluronidase (HAase) (Scheme 20).[38] Polymerization of GlcAβ(1→3)GalNAc oxazoline monomer (R = CH_3) was induced by HAase, to produce the corresponding nonsulfated Ch. The M_n value of "synthetic chondroitin" reached 5.0×10^3, which corresponds to that of naturally occurring Ch. Further, unnatural *N*-propionyl and *N*-acryloyl derivatives of Ch were obtained, with M_n values of 2700 and 3400, respectively. Monomers bearing 2-isopropyl and 2-phenyl groups were not polymerized, probably because of the steric hindrance of the substituents.

Synthesis of structurally well-defined ChS was achieved using HAase as enzyme. Three oxazoline monomers sulfated at C-4 of GalNAc, C-6 of GalNAc, and C-4, 6 of GalNAc were synthesized (Scheme 21).[151] The TSAS monomer sulfated at C-4 was effectively catalyzed by HAase, giving rise to the ChS in good yields. The resulting ChS has sulfonate group exclusvely at C-4 of GalNAc unit, a pure ChS-A. The M_n value ranged from 4.0×10^3 to 1.8×10^4. The other two monomers were not polymerized, and only

Scheme 21



Scheme 22



hydrolyzed disaccharides products were obtained, suggesting that HAase distinguished a sulfate position on GAG chains.

3.2.2.3. Hyaluronidase: A Supercatalyst for Glycosaminoglycan Synthesis. As seen above, hyaluronidase (HAase) showed catalytic activity against various substrate monomers with stereocontrolled and regioselective manner. From the viewpoint of high substrate specificity of the enzyme, these behaviors of the multicatalyst functions are far beyond the "key and lock" theory. Therefore, HAase can be regarded as a supercatalyst for enzymatic polymerization.[152]

3.2.2.4. Keratan. Keratan sulfate (KS) is one of the class of GAGs, having a repeated disaccharide structure of β(1→3)-linked Galβ(1→4)GlcNAc (LacNAc). C-6 in GlcNAc and a portion of C-6 in Gal are sulfonated and the main chain is covalently connected to core proteins through oligosaccharide linkers. KS is hydrolyzed in vivo by the catalysis of keratanase and keratanase II. They are enzymes that hydrolyze Galβ(1→4)GlcNAc and GlcNAcβ(1→3)Gal glycosidic linkages, respectively. Galβ(1→4)GlcNAc(6S) and Gal(6S)β-(1→4)GlcNAc(6S) oxazoline monomers were designed as new TSAS monomers for keratanase II. These two sulfated monomers were recognized by keratanase II and polymerized, producing the corresponding KS oligosaccharide (Scheme 22),[153] suggesting that keratanase II involves an oxazolinium intermediate.

3.3. Unnatural Polysaccharides

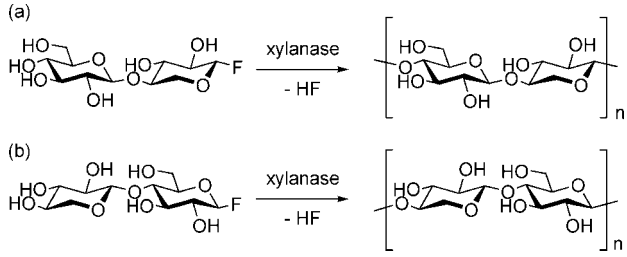
3.3.1. Hybrid Polysaccharides

On the basis of the findings of the wide spectrum in substrate recognition of glycoside hydrolases, syntheses of unnatural polysaccharides composed from different two polysaccharide components were achieved.[154] Such polysaccharides (hybrid polysaccharides) are very difficult to synthesize via conventional chemical synthesis.

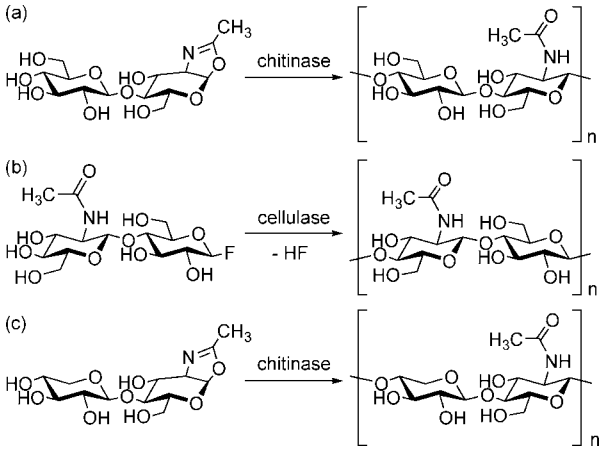
3.3.1.1. Cellulose−Xylan Hybrid Polysaccharide. The first unnatural hybrid polysaccharide synthesized was a cellulose−xylan hybrid polysaccharide.[155] There are two possible monomers to design the hybrid polymer on the basis of the TSAS concept. One is Glcβ(1→4)Xyl-β-fluoride and the other is Xylβ(1→4)Glc-β-fluoride (Scheme 23). Both monomers were polymerized by xylanase from *Trichoderma viride*, giving rise to the corresponding polysaccharide in a 5:1 (v/v) acetonitrile−buffer solution (pH 5.0).

3.3.1.2. Cellulose−Chitin Hybrid and Chitin−Xylan Hybrid Polysaccharides. For the synthesis of a cellulose−chitin hybrid polysaccharide, two kinds of monomers of

Scheme 23



Scheme 24

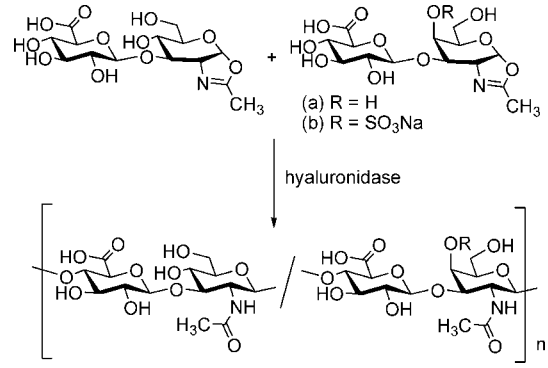


Glcβ(1→4)GlcNAc oxazoline and GlcNAcβ(1→4)Glc-β-fluoride were possible to design on the basis of the TSAS concept for chitinase and cellulase catalysts, respectively (Scheme 24a and b).[156] Both enzymes catalyzed the polymerization (a) via ring-opening polyaddition and (b) via polycondensation, affording the cellulose−chitin hybrid polysaccharide as white precipitates. The M_n values of the products synthesized via chitinase- and cellulase-catalyzed polymerization reached 4.0×10^3 and 2.8×10^3, respectively. Despite a high crystalline structure of both cellulose and chitin, the cellulose−chitin hybrid showed no crystalline structure from the X-ray diffraction measurement.

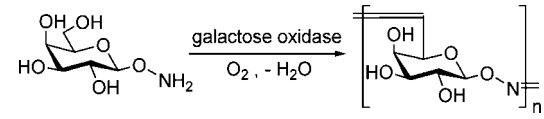
Synthesis of a chitin−xylan hybrid polysaccharide was performed using a Xylβ(1→4)GlcNAc oxazoline TSAS monomer with chitinase catalyst (Scheme 24c).[157] The resulting polysaccharide showed good solubility in water, and no precipitate was observed during the polymerization. From the MALDI-TOF/MS measurement, peaks with the equal molecular mass interval of 335, corresponding to that of the repeating disaccharide unit, were observed. The detectable highest peak was the sugar unit of 60, corresponding to a mass weight larger than 1.0×10^4. No side reactions, such as transglycosylation, were involved in three polymerizations of Scheme 24.

3.3.1.3. Glycosaminoglycan Hybrid Polysaccharides. The synthesis of GAGs hybrids via HAase-catalyzed polymerization was achieved.[158] From the copolymerization of GlcAβ(1→3)GlcNAc oxazoline monomer with GlcAβ(1→3)-GalNAc oxazoline monomer, a copolymer with $M_n = 7.4 \times 10^3$ was obtained in 50% yields (Scheme 25a). Similarly, a copolymer with $M_n = 1.4 \times 10^4$ was produced in 60% yields from the copolymerization using the oxazoline monomer sulfated at C-4 of GalNAc (Scheme 25b).

Scheme 25



Scheme 26



3.3.2. Others

A new carbohydrate polymer was obtained, in which galactose oxidase (GO) catalyzed the oxidative polymerization of β-D-1-*O*-hydroxylamino galactose and related compounds to form a carbohydrate-oxime polymer (Scheme 26).[159] In the reaction, air (oxygen) acted as oxidant. The C-6 CH_2OH group was selectively oxidized first to the C-6 aldehyde intermediate. Then, its polycondensation reaction with $-NH_2$ group led to a polymer having an oxime linkage. In addition to the galactose, other sugars like lactose, melibiose, and arabinose underwent the similar reaction to produce the corresponding polymers in good yields. The M_w values were 4200−8900 ($M_w/M_n = 1.7-2.4$), indicating that approximately 22−25 sugar units were incorporated in these unique polymers.

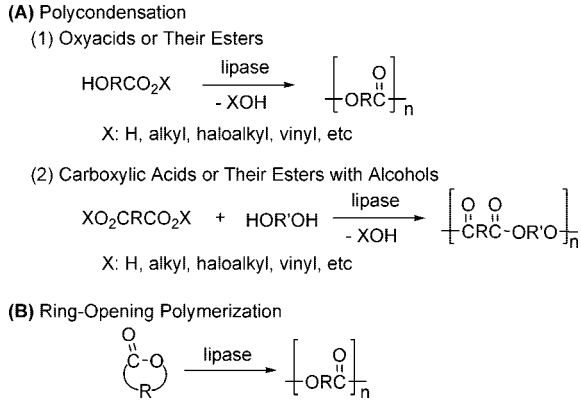
4. Synthesis of Polyesters

Polyesters are in fourth place in living systems following the three major biomacromolecules, nucleic acids (DNA and RNA), proteins (polypeptides), and polysaccharides (see Figure 1).[41] However, polyesters are very important materials that are widely used like poly(ethylene terephthalate) (PET) [an aromatic polyester], poly(butylene succinate), poly(ε-caprolactone) (poly(ε-CL)), and poly(lactic acid) (PLA) [aliphatic polyesters]. Industrially, the former two are produced via polycondensation, and the latter two are produced via ring-opening polymerization.

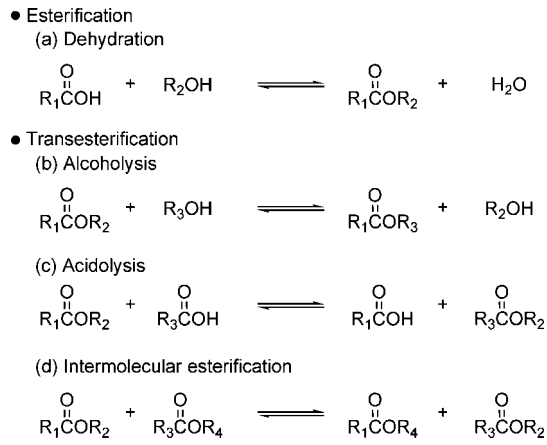
Aliphatic polyesters called poly(hydroxyalkanoate)s (PHAs) were produced via fermentation in the 1980s, and they attracted much attention as biodegradable polymers.[160] In contrast to in vivo enzymatic polymerization in the living cells, in vitro synthesis of polyesters has been developed since the 1980s using a lipase enzyme catalyst.

Lipases (triacylglycerol acylhydrolase, EC 3.1.1.3) catalyze the hydrolysis of fatty acid glycerol esters in vivo with bond-cleaving. As seen in the section 3, where a hydrolase enzyme induced the polymerization to yield polysaccharides, lipase catalyzes polymerization reaction in various solvents to give polyesters in vitro with the bond-forming. Actually, a variety of polyester synthesis reactions have been developed in these two decades.[8,17−20,25,161−165] The reactions proceed via two major polymerization modes: (A) polycondensation between a carboxyl group and an alcohol group, being divided into

Scheme 27



Scheme 28



submodes 1 and 2, and (B) ring-opening polymerization (Scheme 27).[25]

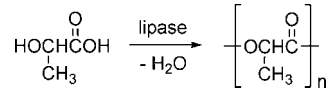
4.1. Polycondensation

The condensation reaction to form an ester is composed from four modes of elemental reactions, esterification (dehydration), and transesterifications (Scheme 28). The reactions are all reversible; therefore, to shift the reaction equilibrium to the product side and to make the reaction proceed faster, the products, like water or an alcohol, are often removed or reduced from the reaction mixture. The lipase-catalyzed polyester synthesis via polycondensation (condensation polymerization) utilizes the reaction of all four modes, the catalysis of which is the reverse direction of the inherent lipase catalysis of hydrolysis.

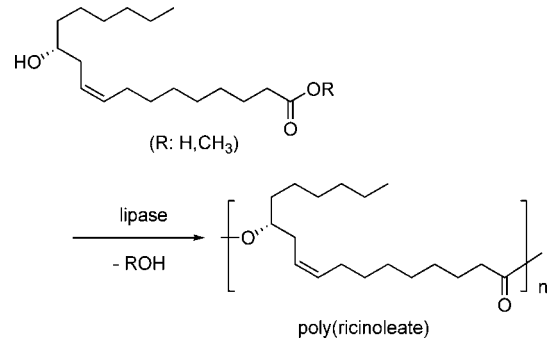
4.1.1. Polycondensation of Oxyacids or Their Esters

4.1.1.1. Via Esterification (Dehydration). Dehydration polycondensation needs neither activation of carboxylic acid groups nor protection−deprotection chemistry, and hence, it is the simplest polycondensation mode. The first paper appeared in 1985, reporting a lipase-catalyzed polycondensation of an oxyacid monomer, 10-hydroxydecanoic acid, in benzene using poly(ethylene glycol) (PEG)-modified lipase soluble in the medium. The degree of polymerization (DP) value of the product was more than 5. The PEG-modified esterase also induced the oligomerization of glycolic acid, the shortest oxyacid.[166] A lipase-catalyzed polymerization of lactic acid gave a low molecular weight poly(lactic acid) (PLA) under a variety of the reaction conditions. The addition

Scheme 29



Scheme 30



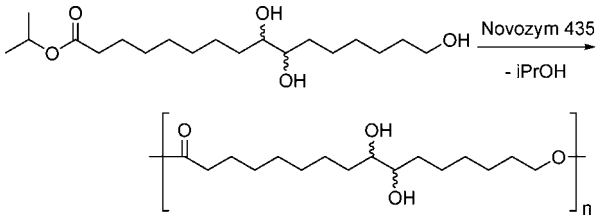
of a small amount of dicarboxylic acid or cyclic anhydrides enhanced the molecular weight (Scheme 29).[167,168]

Dehydration polycondensation of ricinoleic acid, a main component derived from castor oil, proceeded using lipases as catalyst at 35 °C in a hydrocarbon solvent or benzene to give the polymer with molecular weight around 1×10^3 (Scheme 30).[169] Ricinoleic acid was also polymerized via dehydration with immobilized lipase PC (*Pseudomonas cepacia*) catalyst to give the polymer with M_w up to 8 500.[170] Poly(ricinoleate) thus obtained was further applied to develop a new thermosetting elastomer.[171]

Lipase induced the polymerization of 18-hydroxyoctadecanoic acid, 16-hydroxyhexadecanoic acid, and 12-hydroxydodecanoic acid. With catalyst of lipase from *Candida antarctica* (lipase CA or CA lipase B (CALB) immobilized on an acrylic resin commercially called Novozym 435), oligomerization of cholic acid, a hydroxy group at 3-position was regioselectively acylated to give the oligoester with molecular weight of 920.[172] Polyesters of relatively high molecular weight were enzymatically produced from 10-hydroxydecanoic acid and 11-hydroxyundecanoic acid using a large amount of lipase CR (*Candida rugosa*) catalyst (10 weight-folds for the monomer). In the case of 11-hydroxyundecanoic acid, the corresponding polymer with molecular weight of 2.2×10^4 was obtained in the presence of activated molecular sieves.[173] Lipase CA induced the polycondensation of hydrophobic oxyacids efficiently. In the polymerization of 16-hydroxyhexadecanoic acid, 12-hydroxydodecanoic acid, or 10-hydroxydecanoic acid under vacuum at a higher temperature (90 °C) in bulk for 24 h, the degree of polymerization (DP) value was beyond 100, whereas the polyester with lower molecular weight was formed from 6-hydroxyhexanoic acid under the similar reaction conditions.[174] Immobilized CALB (Novozym 435) was an efficient catalyst for the dehydration polycondensation of an oxyacid, *cis*-9,10-epoxy-18-hydroxyoctadecanoic acid isolated from the outer birch bark (*Betula verrucosa*), perfomed in toluene in the presence of molecular sieves at 75 °C to give the polyester with the highest M_w of 20 000 ($M_w/M_n = 2.2$) at a reaction time of 68 h.[175]

Polycondensation copolymerization of 12-hydroxydodecanoic acid with methyl 12-hydroxystearate (both from seed oil) was catalyzed with Novozym 435 in toluene in the presence of molecular sieves at 90 °C. The feed ratio of the monomers and reaction time were varied. During the reaction

Scheme 31



water as well as methanol liberated. After several days, the copolymer was obtained in good yields, having $M_w \approx 1.0 \times 10^5$ showing elasticity and biodegradability.[176]

4.1.1.2. Via Transesterification. Activation of carboxylic acid groups is normally conducted by their esterification. Elemental reactions of transesterification polycondensation are given in b, c, and d in Scheme 28. Polycondensation of methyl 6-hydroxyhexanoate, an oxyacid ester, with lipase catalyst was reported. The polyester with DP up to 100 was synthesized in hexane up to 69 °C for more than 50 days. Porcine pancreas lipase (PPL)-catalyzed polymerization of methyl 5-hydroxypentanoate for 60 days produced the polymer with DP of 29.[177]

Various hydroxyesters, ethyl esters of 3- and 4-hydroxybutyric acids, 5- and 6-hydroxyhexanoic acids, 5-hydroxydodecanoic acid, and 15-hydroxypentadecanoic acid, were polymerized by *Pseudomonas* sp. lipase (lipase PS) at 45 °C to give the corresponding polyesters with molecular weight of several thousands.[178] The enzymatic polycondensation of methyl ricinoleate (R = CH_3 in Scheme 30) by immobilized lipase PC catalyst produced poly(ricinoleate) with high molecular weight ($M_w > 1 \times 10^5$) in bulk in the presence of molecular sieves at 80 °C after 7 days. The polymer was a viscous liquid at room temperature with T_g of −74.8 °C and biodegradable, which was readily cured to give chloroform insoluble cross-linked materials.[170]

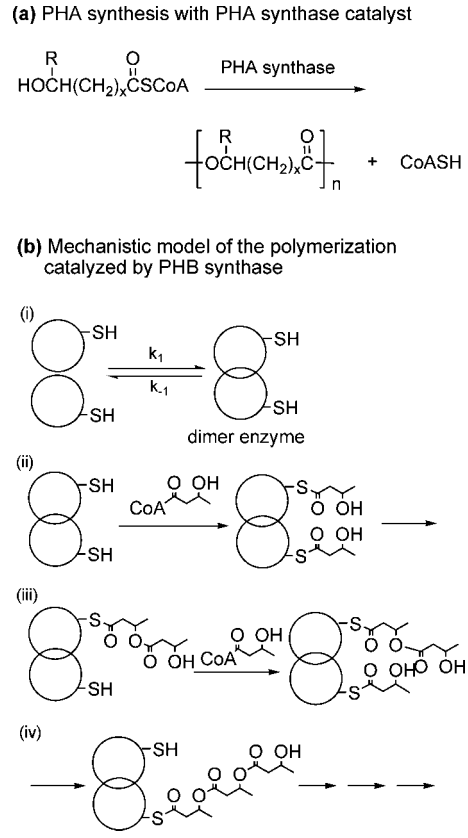
A regioselective polycondensation of isopropyl aleuriteate was achieved by Novozym 435 catalyst, where the only primary alcohol was involved in the reaction at 90 °C in toluene and 2,4-dimethyl-3-pentanol as a cosolvent (Scheme 31). The polymer of M_n 5600 was obtained in 43% yields. Copolymerization of isopropyl aleuriteate with ε-CL gave a random copolymer having M_n up to 10 600 in ~70% yields with the enzyme catalysis.[179]

Structural control of polymer terminal was studied, because terminal-functionalized polymers, typically macromonomers and telechelics, are often used as prepolymers for synthesis of functional polymers. The enzymatic polycondensation of 12-hydroxydodecanoic acid in the presence of 11-methacryloylaminoundecanoic acid conveniently produced the methacrylamide-type polyester macromonomer.[180]

An optically active oligoester was enatioselectively prepared from racemic 10-hydroxyundecanoic acid by lipase CR catalyst. The resulting oligomer was enriched in the (*S*) enantiomer to a level of 60% enantio-excess (ee), and the residual monomer was recovered with 33% ee favoring (*R*) enantiomer.[181] Optically active oligomers (DP < 6) were also synthesized from racemic ε-substituted-ε-hydroxy esters using PPL catalyst. The enantioselectivity increased as a function of bulkiness of the monomer substituent.[182]

Transesterification polycondensation of racemic AB type monomers having a secondary hydroxy group and a methyl ester moiety led to chiral polyesters by iterative tandem catalysis (ITC, see also Schemes 39 and 61). The concurrent actions of an enantioselective acylation catalyst (Novozym 435) and a racemization catalyst (Ru(Shvo)) brought about the high conversion of the racemic monomers to enantio-enriched polymers. AB type monomers used were typically methyl 6-hydroxyheptanoate, methyl 7-hydroxyoctanoate, methyl 8-hydroxynonanoate, and methyl 13-hydroxytetradecanoate. The polycondensation at 70 °C in toluene gave a polyester in high yields having M_n around several thousands with ee higher than 74%.[183]

Scheme 32



An oleic acid-based polyester was prepared via a chemoenzymatic method. First, oleic acid (a C-18 monoene acid from vegetable oils) was epoxidized by lipase catalyst with H_2O_2 oxidant, and then the intermolecular ring-opening addition between the epoxide group and COOH group thermally took place to produce the poly(oleic acid)-based polyester, which was further cross-linked by a diisocyanate compound to give a biodegradable material.[184]

Besides the lipase catalysts, the in vitro enzymatic polymerization by using a bacterial synthase or polymerase (PHA synthase; EC 2.3.1) as catalyst is mentioned here as another in vitro enzymatic polyester synthesis. Poly(hydroxyalkanoate)s (PHAs) are well-known as biodegradable polyesters produced commercially via fermentation.[160] Approximately 60 different PHA synthases have been isolated and characterized.[185,186] The reaction type of the PHA synthase-catalyzed polymerization is of polycondensation. Many species of bacteria produce PHAs as intracellular energy and carbon reserve materials.[185,187]

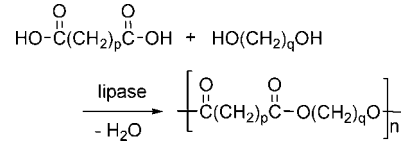
PHAs include poly[(*R*)-3-hydroxybutylate] [P(3HB)], poly[(*R*)-3-hydroxyvalerate] [P(3HV)], poly[4-hydroxybutylate] [P(4HB)], etc. The in vitro synthesis of a PHA via the polymerization of (*R*)-3-hydroxybutyryl coenzyme A (3HB-CoA) was reported first with using a PHB synthase from *Ralstonia eutropha* (*R. eutropha*) as catalyst (a general scheme in Scheme 32a; R = CH_3, $x = 1$).[188,189] The

monomers, CoA thioester derivatives of 3HB and 3HV, were chemically prepared and polymerized in an aqueous solution at room temperature by the catalysis of the purified, recombinant polymerase from *R. eutropha*.[190,191] Homopolymerization of 3HB-CoA gave P(3HB) with high molecular weight M_n of 4.21×10^6 (M_w 7.41×10^6), indicating a close value of the monomer-to-polymerase ratio (2×10^4). This shows a "living nature" of the polymerization. In fact, a block copolymer of P(3HB)-*b*-P(3HV) was obtained by the two step sequential copolymerizations of 3HB-CoA and then 3HV-CoA. In the batch copolymerization of the two monomers, random to somewhat blocky copolymers were produced.

In the PHA production catalyzed by purified *R.eutropha* PHA synthase from recombinant cells, the substrate reactivity was examined by the kinetic study.[192] Monomer 3HB-CoA showed a higher reactivity than monomer 3HV-CoA. The change of hydroxy group from the β in 3HB-CoA to the γ position (4HB-CoA) caused a dramatic decrease in the binding ability of the latter. A detailed mechanistic study on PHB synthase from *R.eutropha* led to a revised model (Scheme 32b).[190] Purified PHB synthase can exist in equilibrium with monomer and dimer (step i). The active enzyme is a homodimer; in spite of two identical thiol groups of Cys_{319} responsible for the catalysis[193] the dimer shows only a single catalytic site. The initiation step involves the binding of two monomer substrates followed by thioacylation of the two thiol groups on the two Cys_{319} (step ii). The esterification (first elongation) reaction could occur by the reaction of a β-hydroxy group on either monomer with the thioester group to form a two ester unit and a free thiol group (steps ii−iii). In the next elongation, the free thiol group accepts another monomer and the process can continue (steps iii−iv). This model suggests that two identical thiol groups form one catalytic site and both can be used in turn as donor and acceptor in the polymerization.

The in vitro polymerization of 3HB-CoA catalyzed by the purified PHA synthase from *Chromatium vinosum* provided a suitable model for verifying and extending the examination results of PHA biosynthesis in vivo. From the polymerization behaviors of 3HB-CoA by *Chromatium vinosum* PHB catalyst, it was discussed as a different class of *R. eutrophus* PHB synthase.[194] PHA synthase from *R. eutropha* was immoibilized onto several solid surfaces through a transition-metal complex, Ni^{2+}−nitrilotriacetic acid. The immobilized PHA synthase catalyzed the surface-initiated enzymatic polymerization of 3HB-CoA, forming a polyester of P(3HB) film with a uniform thickness on the surface. Various substrate surfaces like agarose beads, Si/SiO_2 glass, aluminum oxide, and gold can be modified.[195] Surface modification of two hydrophobic substrates, highly oriented pyrolytic graphite (HOPG) and an alkanethiol self-assembled monolayer (SAM), was conducted through PHA synthase (from *R. eutropha*)-catalyzed polymerization of (*R*)-monomers of 3HB-CoA and 3HV-CoA.[196] The resulted polyester-modified surfaces were analyzed by atomic force microscopy (AFM) and quarz crystal microbalance (QCM), revealing the PHA film thickness of 1−6 nm on HOPG and SAM surfaces. It was suggested that PHA synthase enzyme is to be located near a hydrophobic substrate surface to give thin film formation.

Scheme 33



4.1.2. Polycondensation of Carboxylic Acids or Their Esters with Alcohols

4.1.2.1. Via Esterification (Dehydration). The simplest polycondensation mode of dehydration is given in Scheme 33.

It was in 1984 that the first paper on the lipase-catalyzed polymerization reported an *Aspergillus niger* lipase (lipase A)-catalyzed dehydration polycondensation of several free dicarboxylic acids in the presence of an excess amount of a diol, giving rise to oligomeric polyesters.[197] For example, a dehydration polycondensation of adipic acid and 1,4-butanediol in diisopropyl ether gave a polyester with a degree of polymerization (DP) of 20 ($p = q = 4$, Scheme 33).[198] Higher molecular weight polyesters were enzymatically obtained by polycondensation of sebacic acid and 1,4-butanediol ($p = 8$, $q = 4$ in Scheme 33) under vacuum. In the *Mucor miehei* lipase (lipase MM)-catalyzed polymerization in hydrophobic solvents with high boiling points, such as diphenyl ether and veratrole, the molecular weight of polyesters from various combinations of diacids and glycols reached the value higher than 4×10^4.[199−201]

In the lipase-catalyzed dehydration polycondensation, effects of substrates and solvent on the ester-chain formation, polydispersity, and end-group structure were examined in detail. Diphenyl ether was a preferred solvent for the polycondensation of adipic acid ($p = 4$) and 1,8-octanediol ($q = 8$) giving M_n of 28 500 (48 h, 70 °C). The reactions involving monomers having longer alkylene chain length of diacids (sebacic and adipic acids) and diols (1,8-octanediol and 1,6-hexanediol) showed a higher reactivity than the reactions of shorter chain length diacids and 1,4-butanediol. A reaction using diphenyl ether solvent gave rather higher molecular weight polyester than a bulk reaction.[202] Under vacuum, the molecular weight was greatly enhanced, leading the equilibrium toward the product polymer side; a polyester with molecular weight of 2×10^4 was obtained by the lipase-catalyzed polymerization of sebacic acid and 1,4-butanediol in diphenyl ether or veratrole under reduced pressure.[203] In ionic liquids, a dehydration polycondensation gave effectively the product polyester with lipase catalysis.[204]

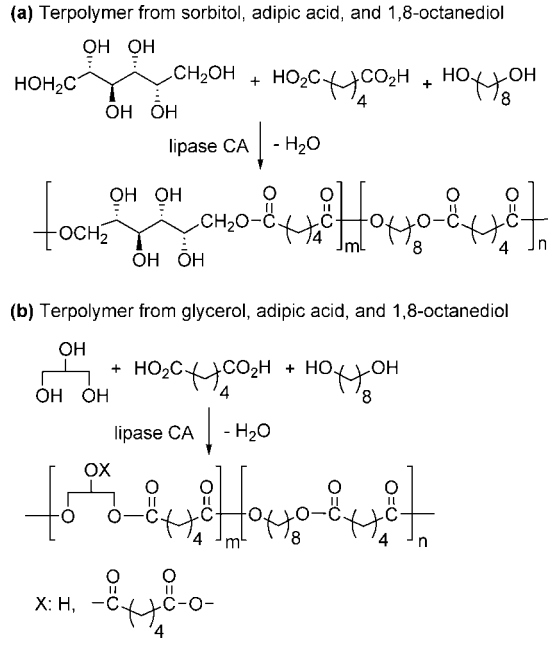
In a solvent-free system, lipase CA (CALB) efficiently catalyzed the polycondensation of dicarboxylic acids and glycols under mild reaction conditions at 60 °C, despite the initial heterogeneous mixture of the monomers and catalyst. Methylene chain length of the monomers greatly affected the polymer yield and molecular weight. The polymer with molecular weight higher than 1×10^4 was obtained by the reaction under reduced pressure.[205,206] CALB was covalently immobilized onto epoxy-activated macroporous poly(methyl methacrylate) Amberzyme beads and epoxy-activated nanoparticles (nanoPSG) with a poly(glycidyl methacrylate) outer region. In bulk, Amberzyme-CALB catalyzed polycondensation between glycerol (0.1 equiv), 1,8-octanediol (0.4 equiv), and adipic acid (0.5 equiv) at 90 °C for 24 h gave polyesters of $M_w \approx 4.0 \times 10^4$, and the catalyst showed a better stability in recycled use over Novozyeme 435.[207]

In the polymerization of adipic acid and 1,6-hexanediol, the loss of the enzymatic activity was small during the polymerization, whereas less than half of the activity remained in using glycols with methylene chain length less than 4.[208,209] A scale-up experiment produced the polyester in more than 200 kg yield, claiming a large potential as an environmentally friendly synthetic process of polymeric materials because of the mild reaction conditions without using organic solvents and toxic catalysts.[208]

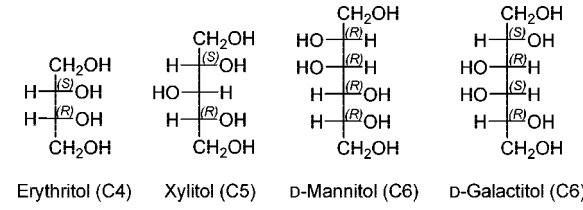
A dehydration reaction is generally conducted in nonaqueous media. Since the product water of the dehydration is in equilibrium with starting materials, the solvent water disfavors the dehydration to proceed in an aqueous medium because of the "law of mass action". Nevertheless, lipase catalysis enabled a dehydration polycondensation of a dicarboxylic acid and a glycol in water at 45 °C to afford a polyester in good yields.[210,211] Lipases CA and other lipases were active for polycondensation of sebacic acid and 1,8-octanediol. In the polymerization of an α,ω-dicarboxylic acid and a glycol, the polymerization behavior was greatly depending on the methylene chain length of the monomers. The polymer was obtained in good yields from 1,10-decanediol, whereas no polymer formation was observed from 1,6-hexanediol, suggesting that the combination of the monomers with appropriate hydrophobicity is needed for the polymer production by dehydration. This finding of dehydration in water is a new aspect in organic chemistry.

Dehydration polymerization was extended to use polyols including sugar components. For example, the direct polycondensation between adipic acid and sorbitol in bulk was carried out with Novozym 435 catalyst (10 wt % relative to the monomers) at 90 °C for 48 h. The polymer poly(sorbityl adipate) was water-soluble. The M_n and M_w values were 10 880 and 17 030, respectively. In the polymer, sorbitol was esterified at primary alcohol group of 1- and 6-positions with high regioselectivity (85 ± 5%). In place of sorbitol, glycerol was employed; however, the M_n and M_w values were lower, 2500 and 3700, respectively. To obtain a water insoluble sorbitol copolyester, adipic acid, 1,8-octanediol, and sorbitol (molar ratio 50:35:15) were ter-polymerized at 90 °C for 42 h (Scheme 34a). The methanol-insoluble part (80%) had M_w of 1.17×10^5. From the solubility data, the polymer was considered to have few cross-links. Another terpolymerization of adipic acid, 1,8-octanediol, and glycerol, in the ratio 50:40:10 mol %, was performed in bulk at 70 °C, to give a polyester (Scheme 34b). The product polymer showed the monomers ratio in the 50: 41: 9, respectively, and the values for M_w and M_w/M_n of 75 600 and 3.1, respectively. The product contained 90% methanol-insoluble parts, showing few cross-links. The selectivity at glycerol primary alcohol sites was only 66%, therefore, the product was highly branched; 27% of glycerol units were for branched sites.[212] Physical properties of the resulting polyesters containing sorbitol or glycerol were characterized in detail.[213] Lipase CA catalyzed bulk dehydration polycondensation of the monomers, adipic acid (A_2), 1,8-octanediol (B_2), and glycerol ($B'B_2$), gave hyperbranched polyesters at 70 °C for 42 h. With monomer feed molar ratio (A_2 to B_2 to $B'B_2$) 1.0: 0.8: 0.2, linear copolyesters were formed during the first 18 h, and extending the reaction time to 42 h gave hyperbranched copolymers with dendritic glycerol units. The regioselectivity for esterification at the primary glycerol positions ranged from 77 to 82%. Variation of glycerol in the monomer feed gave copolymers with degree of branching from 9 to 58%.[214] A similar dehydaration polycondensation to produce terpolymers was conducted by using monomers, adipic acid (A_2), 1,8-octanediol (B_2), and trimethyrolpropane (TMC, B_3) with lipase CA catalyst in bulk at 70 °C for 42 h. As an example, a hyperbranched copolyester with 53% TMC adipate units was obtained in 80% yields, with M_w 14 100 ($M_w/M_n = 5.3$).[215]

Scheme 34



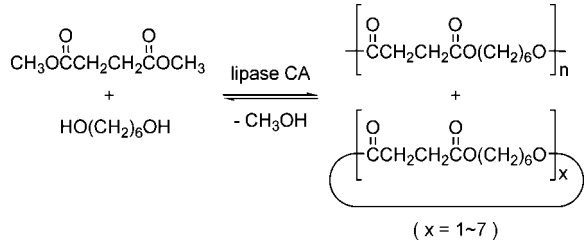
Scheme 35



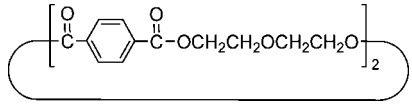
Natural sugar polyols were used to prepare "sweet polyesters". The polyols employed were C4-carbon, erythritol, C5-carbon, xylitol and ribitol, and C6-carbon, mannitol, glucitol, and galactitol; four structures among them are shown in Scheme 35. The ter-polymeriztion by Novozym 435 catalyst was performed in bulk for the combination of a polyol, adipic acid, and 1,8-octanediol under vacuum at 90 °C. The vacuum control during the reaction was very important to yield a high molecular weight polyester. The M_w value of the product polyol-polyester ranged from 1.1×10^4 (D-galactitol) to 7.3×10^4 (D-mannitol), having a branching structure. Primary alcohol groups are more reactive than the secondary groups, which were also reacted.[216]

A dehydration polycondensation was effectively catalyzed by cutinase (EC 3.1.1.74).[217] Glycols like 1,4-butanediol, 1,6-hexanediol, 1,8-octanediol, and 1,4-cyclohexanedimethanol (1,4-CHDM), and diacids like adipic acid, succinic acid, suberic acid, and sebacic acid, were combined for the polycodensation with 1% w/w enzyme at 70 °C for 48 h under vacuum. In all reactions the monomers were consumed quantitatively. With fixing the adipic acid component, polyesters from 1,4-butanediol, 1,6-hexanediol, and 1,8-octanediol showed M_n values of 2700, 7000, and 12 000, respectively. With fixing the 1,4-CHDM component, poly-

Scheme 36



Scheme 37



esters from succinic acid, adipic acid, suberic acid, and sebacic acid possessed M_n values of 900, 4000, 5000, and 19 000 for these C4, C6, C8, and C10 diacids, respectively. For both glycols and diacids, there was a tendency that the higher the hydrophobicity, higher the molecular weight of the product polyester.

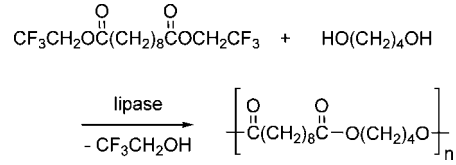
Furthermore, the reaction of a linear polyanhydride, such as poly(azelaic anhydride), and a glycol, such as 1,8-octanediol, was induced with lipase CA catalyst involving the dehydration to give a polyester with molecular weight of several thousands.[218]

4.1.2.2. Via Transesterification. Transesterification polycondensation normally needs activation of carboxylic acid groups, whose elemental reactions are shown in b, c, and d in Scheme 28. The activation is conducted ordinarily by esterification of the acid group. In the early studies, alkyl or haloalkyl esters and later vinyl esters have been often used.

In 1989, a lipase-catalyzed high enantioselective polymerization was reported; the reaction of bis(2,2,2-trichloroethyl) *trans*-3,4-epoxyadipate with 1,4-butanediol in anhydrous diethyl ether using porcine pancreas lipase (PPL) catalyst gave a highly optically active polyester. The feed molar ratio of the diester to the diol was adjusted to 2:1 so as to produce the (−)-polymer resulting in enantiomeric purity >96%. The molecular weight was estimated as 5300. The unchanged (+)-monomer was shown to have an enantiomeric purity higher than 95%.[219]

Polycondensation between dimethyl succinate and 1,6-hexanediol catalyzed by lipase CA in toluene at 60 °C reached the ring−chain (cyclic-linear structure) equilibrium of the product polymer. Adsorption of methanol by molecular sieves or elimination of methanol by nitrogen bubbling shifted to the thermodynamic equilibrium. Polyesters with the molecular weight about several thousands were prepared from α,ω-alkylene dicarboxylic acid dialkyl esters, and regardless of the monomer structure, cyclic oligomers were formed (Scheme 36).[220] In the polymerization of dimethyl terephthalate and diethylene glycol catalyzed by lipase CA in toluene for producing a terephthalate polymer, the distribution of the macrocyclic species obeyed the Jacobson−Stockmayer theory, in terms of ring−chain equilibrium.[221] A cyclic dimer (C2 macrocyclic, Scheme 37) was selectively formed. The C2 content (%) in the products was dependent on reaction temperature and reaction time: at 50 °C, 2, 24, and 61% after 4, 8, and 24 h, respectively, and at 80 °C, 68, 80, and 99% after 4, 8, and 24 h, respectively. It was considered that among several factors the selective C2 cyclic formation is ascribed to the presence of a driving force

Scheme 38



because of a $\pi-\pi$ stacking of the aromatic rings together with a relative flexibility of the diol segment.[222] Protease was effective for aromatic polyester synthesis; a terephthalic acid diester and 1,4-butanediol produced oligomers.[223]

Dicarboxylic acid dimethyl esters having unsaturated C_{18}, C_{20}, C_{26} alkylene chains were epoxidized via chemoenzymatical oxidation with hydrogen peroxide/methyl acetate with lipase CA (Novozym 435) catalyst. Polycondensations of these dimethyl esters with a diol by the lipase catalysis gave the linear unsaturated and epoxidized polyesters with molecular weight of 1950−3300 and melting point of 47−75 °C from a 1,3-propanediol substrate, and molecular weight of 7900−11600 and melting point of 55−74 °C from a 1,4-butanediol substrate.[224] Lipase (Novozym 435)-catalyzed synthesis of poly(butylene succinate) (PBS) via polycondensation was achieved using a monophasic reaction mixture of dimethyl succinate and 1,4-butanediol in bulk and in solution. Diphenyl ether was a preferred solvent to give a higher molecular weight PBS; at 60, 70, 80, and 90 °C after 24 h, M_n values of PBS were 2000, 4000, 8000, and 7000, respectively. The reaction at 95 °C after 21 h gave PBS with M_n value of 38 000.[225]

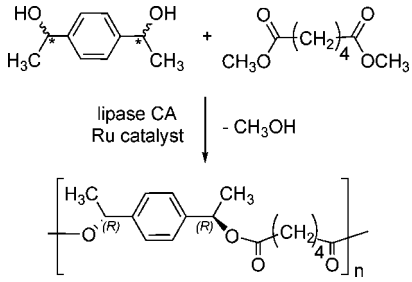
Ionic liquids, as a green solvent, were found to act as a good medium for lipase-catalyzed production of polyesters. The lipase CA-catalyzed polycondensation of diethyl adipate with 1,4-butanediol efficiently proceeded in 1-butyl-3-methylimidazolinium tetrafluoroborate or hexafluorophosphate under reduced pressure at 60 °C to produce the polyester with M_n up to 1 500 after 72 h in 91% yields.[204] Polycondensation of diethyl sebacate or dimethyl adipate and 1,4-butanediol in 1-butyl-3-methylimidazolinium hexafluorophosphate took place at 70 °C or even at room temperature in the presence of lipase PC or Novozym 435.[226,227]

Transesterifications using lipase catalyst are often very slow because of the reversible nature of the reactions. To shift the equilibrium toward the product polymer more effectively, activation of esters was conducted by using a halogenated alcohol like 2-chloroethanol, 2,2,2-trifluoroethanol, and 2,2,2-trichloroethanol (Scheme 38). Compared with methanol or ethanol, they increased the electrophilicity of the acyl carbonyl and avoided significant alcoholysis of the products by decreasing the nucleophilicity of the leaving alkoxy group.

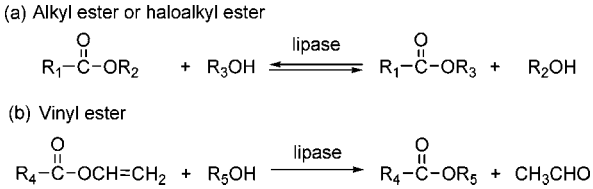
Lipase PF (*Pseudomonas fluorescens*)-catalyzed reaction of bis(2-chloroethyl) succinate and 1,4-butanediol carried out in a mixed solvent of diisopropyl ether and chloroform at 37 °C gave the polyester with the highest M_n of 1 570.[200] Polycondensation of bis(2,2,2-trichloroethyl) glutarate and 1,4-butanediol proceeded with PPL catalyst at room temperature in diethyl ether to produce the polyesters with molecular weight of 8.2×10^3.[228]

Polycondensation of bis(2,2,2-trifluoroethyl) adipate with sucrose catalyzed by an alkaline protease (EC 3.4) from *Bacillus* sp. resulted in an alternating linear polyester soluble in water and polar organic solvents with M_n value around 1600 in low yields. It was claimed that sucrose reacted regioselectively at 6- and 1′- positions and behaved like a

Scheme 39



Scheme 40



diol; cross-linking did not take place.[229] In the PPL-catalyzed polymerization of bis(2,2,2-trifluoroethyl) glutarate with 1,4-butanediol in 1,2-dimethoxybenzene, a periodical vacuum method for removing 2,2,2-trifluoroethanol from the reaction mixture increased the molecular weight to nearly 4×10^4.[230] Also, the vacuum technique was effective to increase the molecular weight. The lipase-catalyzed polycondensation between bis(2,2,2-trifluoroethyl) sebacate and aliphatic diols was performed at 37 °C. The elimination of the product 2,2,2-trifluoroethanol was critical for obtaining the higher molecular weight polyesters; the polyester from 1,4-butanediol reached the highest M_w of 46 400 (Scheme 38).[201]

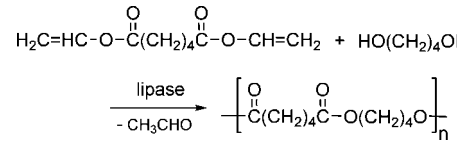
In a supercritical fluoroform solvent, polycondensation of bis(2,2,2-trichloroethyl) adipate with 1,4-butanediol using PPL catalyst proceeded, giving rise to the polymer with molecular weight of several thousands.[231]

A dynamic kinetic resolution (DKR) method was used to synthesize an optically active polyester from a racemic monomer via polycondensation.[232] A mixture of stereoisomers of a secondary diol, α,α′-dimethyl-1,4-benzenedimethanol, was enzymatically polymerized with dimethyl adipate (Scheme 39). Because of the enantioselectivity of lipase CA, only the hydroxy groups at the (*R*) center are preferentially reacted to form the ester bond with liberation of methanol. The reactivity ratio was estimated as $(R)/(S) = \sim 1 \times 10^6$. In situ racemization from the (*S*) to the (*R*) configuration by Ru catalysis allowed the polymerization to high conversion, that is, the enzymatic polymerization and the metal-catalyzed racemization occurred concurrently. The DKR polymerization was carried out for 4 days; during the reaction, molecular weight increased to 3000−4000, and the optical rotation of the reaction mixture increased from −0.6° to 128°. Eventually all product polymers will be end-capped with (*S*) stereocenters (chain-stoppers), and the racemization is occurring at the end-groups of growing polymer chains.

An irreversible process was developed by using a vinyl ester for the lipase-catalyzed acylation, where the product of vinyl alcohol tautomerizes to acetaldehyde (Scheme 40).[233] The reaction of an alcohol with a vinyl ester proceeds much faster than with an alkyl ester or a haloalkyl ester to form the desired product in higher yields.

A divinyl ester was employed for the first time as the activated acid form in the enzyme-catalyzed polyester synthesis. The lipase PF-catalyzed polycondensation of

Scheme 41



divinyl adipate and 1,4-butanediol was performed in 1994 at 45 °C in diisopropyl ether for 48 h to afford a polyester with M_n of 6.7×10^3 (Scheme 41), whereas the use of adipic acid and diethyl adipate did not produce the polymeric materials under the similar reaction conditions. As a diol, ethylene glycol, 1,6-hexanediol, and 1,10-decanediol were also reacted to give the corresponding polyester with molecular weight of several thousands.[234] The same polycondensation of divinyl adipate and 1,4-butanediol with lipase PC (*Pseudomonas cepacia*) catalyst produced the polyester with M_n of 2.1×10^4.[235] By variation of the molar ratio of the divinyl ester and the glycol, a macromonomer having the glycol, the dicarboxylic acids, or the acid-alcohol end structure are to be obtained. The reaction mechanism is considered similarly as that of the ring-opening polymerization of lactones to involve an acyl-enzyme intermediate (enzyme-activated monomer, EM, see section 4.2.1.4) from lipase and the vinyl ester with liberating acetaldehyde.

During the lipase-catalyzed polymerization of divinyl esters and glycols, there was a competition between the enzymatic transesterification and hydrolysis of the vinyl end group, resulting in the limitation of the polymer growth.[236] A batch-stirred reactor was developed to minimize temperature and mass-transfer effects. Using the reactor, the polycondensation became very fast; poly(1,4-butylene adipate) with the molecular weight of 2.3×10^4 was obtained within 1 h at 60 °C.[237]

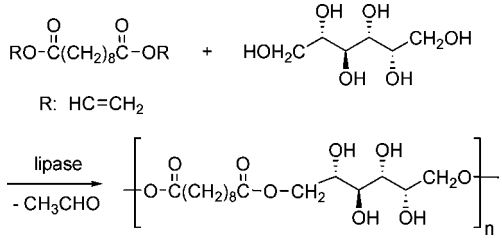
A combinatrial approach was applied for biocatalytic production of polyesters. A library of polyesters was synthesized in 96 deep-well plates from a combination of divinyl esters and glycols with lipases of different origin. In the screening, lipase CA was the most active biocatalyst for the polyester production. As an acyl acceptor, 2,2,2-trifluoroethyl esters and vinyl esters were examined and it was claimed that the former produced the polymer of higher molecular weight.[238]

Supercritical carbon dioxide ($scCO_2$) was shown to be a good solvent for the lipase-catalyzed polycondensation of divinyl adipate and 1,4-butanediol. Quantitative consumption of both monomers was achieved to give the polyester with M_n of 3.9×10^3.[239]

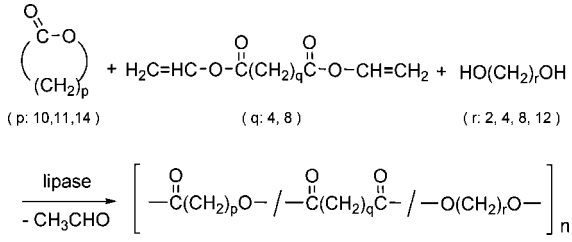
In an ionic liquid, such as 1-butyl-3-methy-imidazolium tetrafluoroborate ([bmim][BF_4]), a similar polycondensation between diethyl adipate or diethyl sebacate and 1,4-butanediol gave the polyester having M_n up to 1500 in good yields. Since the ionic liquid is nonvolatile, ethanol was removed under vacuum during the reaction.[204] Lipase CA-catalyzed polycondensation of dimethyl adipate or dimethyl sebacate with 1,4-butanediol was performed in an ionic liquid such as [bmim][BF_4], [bmim][PF_6], and [bmim][$(CF_3SO_2)_2N$] at 70 °C for 24 h to give a higher molecular weight polyester, M_n reaching several thousands. Using ionic liquids as solvent involves the wide range of tunability of solvent hydrophilicity and monomer solubility for the reaction.[227]

Aromatic polyesters were produced efficiently by the lipase CA-catalyzed polycodensation of aromatic diacid divinyl esters. Divinyl esters of isophthalic acid, terephthalic acid,

Scheme 42



Scheme 43



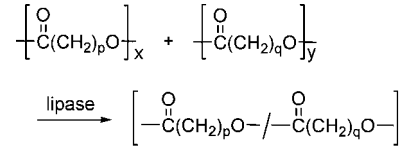
and *p*-phenylene diacetic acid were polymerized with various glycols to give polyesters containing aromatic moiety in the main chain with the highest M_n of 7200 in heptane at 60 °C for 48 h.[240] Enzymatic polycondensation of divinyl esters with aromatic diols also afforded the aromatic polyesters.[241]

Regioselctive polycondensation of polyols was achieved with lipase catalysis by using divinyl esters. Triols such as glycerol were regioselectively polymerized at a primary hydroxy group with divinyl adipate by lipase catalyst to produce a linear polyester linked through mainly primary hydroxy group having also a secondary hydroxy group (5−10%) in the main chain. The polymer contained pendant hydroxy groups, with no evidence of network structure, having M_w value from ∼3000 to 14 000.[242] The reaction of divinyl sebacate and glycerol with lipase CA catalyst produced water-soluble polyesters with M_w up to 2700 in bulk at 60 °C for 8 h under argon. The chloroform-soluble part with M_w of 19 000 was isolated in 63%, which indicated the regioselectivity of primary OH/ secondary OH ratio of 74/26. At 45 °C, however, the regioselectivity was perfectly controlled to give a linear polymer consisting exclusively of 1,3-glyceride unit.[243] The lipase CA catalysis gave a reduced sugar-containing polyester regioselectively from divinyl sebacate and sorbitol, in which sorbitol was exclusively acylated at the primary alcohol of 1- and 6-positions in acetonitrile at 60 °C for 72 h (Scheme 42). Mannitol and *meso*-erythritol were also regioselectively polymerized with divinyl sebacate.[244]

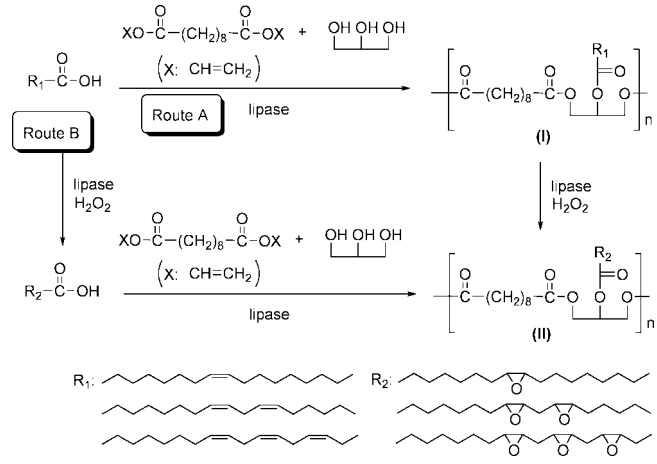
Lipase-catalyzed terpolymerization of divinyl esters, glycols, and lactones produced ester terpolymers with M_n higher than 1×10^4 (Scheme 43). Lipases showed high catalytic activity for the terpolymerization involving both polycondensation and ring-opening polymerization manners simultaneously in one-pot to produce ester terpolymers, without involving homopolymer formation.[245] A similar terpolymerization was performed using three kinds of monomers, ω-pentadecalactone, diethyl succinate, and 1,4-butanediol, by CALB catalyst desirably at 95 °C via a two-stage vacuum technique. The polymerization was examined under various reaction conditions and the product terpolyester reached $M_w = 77\,000$ with M_w/M_n between 1.7 and 4.0.[246]

The above results accord with the frequent occurrence of an intermolecular transesterification between the resulting polyesters during the polymerization; from a mixture of two

Scheme 44



Scheme 45



homopolyesters a copolyester was obtained by lipase catalysis (Scheme 44).[247]

New cross-linkable polyesters were prepared by the lipase-catalyzed polycondensation of divinyl sebacate with glycerol in the presence of an unsaturated higher fatty acid such as linoleic acid and linolenic acid obtained from renewable plant oils (Route A, Scheme 45). Product polyester **I** is biodegradable and contains an unsaturated fatty acid moiety in the side chain. Curing of **I** proceeded by oxidation with cobalt naphthenate catalyst or thermal treatment gave a cross-linked transparent film. Biodegradability of the film obtained was verified by biochemical oxygen demand (BOD) measurement.[248,249] Furthermore, epoxide-containing polyesters were enzymatically synthesized via two routes using unsaturated fatty acids (Routes A and B, Scheme 45). In Route A, **I** was enzymatically epoxidized to give **II**, and in Route B, the epoxidization of the fatty acid was first conducted and lipase-catalyzed polycondensation of the product was performed to produce **II**.[250] Curing of **II** proceeded thermally, yielding transparent polymeric films with high gloss surface. Pencil scratch hardness of the film was enhanced, compared with that of the cured film from **I**. The obtained film showed good biodegradability in the activated sludge test.

A cross-linkable group can also be a mercapto group. Direct lipase CA-catalyzed polycondensation of 1,6-hexanediol and dimethyl 2-mercaptosuccinate at 70 °C in bulk gave an aliphatic polyester having free pendant mercapto groups with $M_w = 14\,000$ in good yields. The mercapto group content could be controlled by copolymerization with other monomers. The polyester was readily cross-linked by the air-oxidation via the disulfide linkage formation in dimethyl sulfoxide.[251]

4.2. Ring-Opening Polymerization

Among the polymerization modes in Scheme 27, ring-opening polymerization (ROP) has been most extensively studied for the polyester synthesis. Typical examples of cyclic monomers studied so far by an enzyme catalyst are shown in Figure 12.

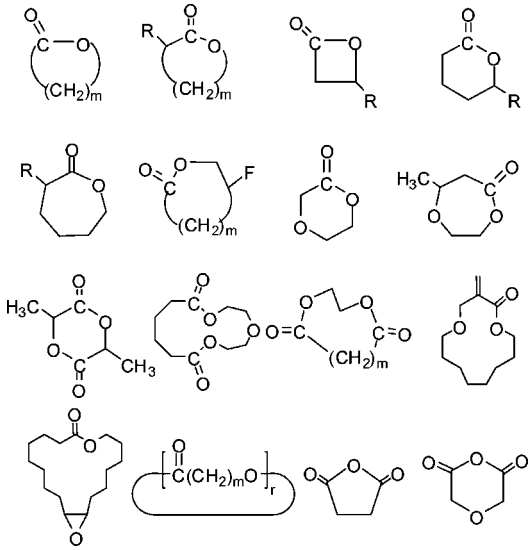
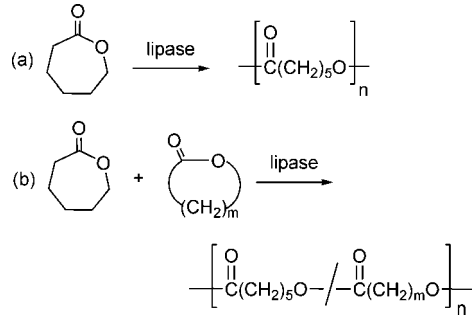


Figure 12. Examples of cyclic monomers for enzyme-catalyzed polyester synthesis.

Scheme 46

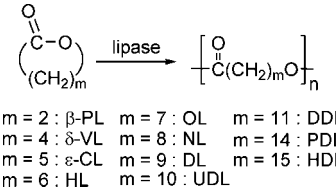


4.2.1. Ring-Opening Polymerization of Cyclic Esters (Lactones)

4.2.1.1. Lipase Catalyst and Monomers. In 1993, lipase-catalyzed ROP was first found for ε-caprolactone (ε-CL, 7-membered) (Scheme 46a) and δ-valerolactone (δ-VL, 6-membered) by two independent groups.[177,252,253] ROP of ε-CL by lipase PF (*Pseudomonas fluorescens*) in bulk at 75 °C for 10 days gave poly(ε-CL) in 92% yields, having molecular weight M_n of 7700 with $M_w/M_n = 2.4$. Similarly, δ-VL yielded at 60 °C poly(δ-VL) having $M_n = 1900$ with $M_w/M_n = 3.0$.[252] Other lipases like *Candida cylindracea* (lipase CC) and porcine pancreas lipase (PPL) were also active for ROP of the monomers. These polyesters possessed the terminal structure of a carboxylic acid group at one end and a hydroxy group at the other, indicating that the ROP was initiated by water molecule and terminated also by water, when nothing was added. Catalyst activity was examined with using the ROP of ε-CL for lipases from *Candida antarctica* B (CALB), *Rhizomucor meihei* (lipase RM), *Candida rugosa* (lipase CR), and lipase PF, in the supported or free form.[254]

Enzymatic ring-opening copolymerization by lipase PF catalyst between ε-CL and δ-VL was achieved in bulk at 60 °C for 10 days, giving rise to a copolyester with an M_n value of 3700 with $M_w/M_n = 2.9$, with a random copolyester structure (Scheme 46b, $m = 4$). Copolymerization of ε-CL with other lactones, such as 15-pentadecanolactone (PDL) ($m = 14$ in Scheme 46b) and D-lactide, was also achieved.[253] By using PPL as catalyst and methanol as initiator, ε-CL was polymerized in hexane at 45 °C for up to 26 days to complete monomer

Scheme 47



conversion, affording poly(ε-CL) and dilactone.[177] Following these works, various lactones (cyclic esters) of different ring size, unsubstituted and substituted, and also other cyclic monomers, have been extensively studied for ring-opening polymerization and copolymerization.[8,17−20,163,255−258]

Scheme 47 shows unsubstituted 4- to 17-membered lactones so far polymerized by lipase catalyst for ROP.[259−263] All monomers showed a good ROP reactivity catalyzed by lipase. The polymerization can be performed in bulk, in an organic solvent or in other solvents.

Catalytic activity for ROP of lactone monomers have been examined on a variety of lipase from different origins, such as *Pseudomonas fluorescens* (lipase PF), *Candida cylindracea* (lipase CC), porcine pancreas lipase (PPL), *Aspergillus niger* (lipase A), *Candida rugosa* (lipase CR), *Penicillium roqueforti* (lipase PR), *Pseudomonas cepacia* (lipase PC), *Mucor miehei* (lipase MM), and *Rhizopus japanicus* (lipase RJ). Among others, *Candida antarctica* (lipase CA, CALB, or Novozym 435) has been most often used due to the increased activity recently.[17,18,163,255,259−262]

A 7-membered lactone of ε-CL is the most extensively studied among various lactone monomers ($m = 5$ in Scheme 47).[163,177,252,253,259−262,264−277] Reaction parameters in ROP of ε-CL were examined by using PPL catalyst; the polymerization at 65 °C in heptane after 4 days produced poly(ε-CL) with $M_n = 2300$. With addition of butanol, both butanol and water were involved in the initiation reaction.[264] Lipase CA was found as the most effective for the polymerization of ε-CL.[278,279] For example, the catalyst amount was reduced to 1%, which is compared with the reported systems of 20 to 50% of lipase from other origins. The reaction time could be reduced from 10 days to less than 10 h in bulk at 60 °C. The high catalytic activity was observed also for 12- and 13-membered lactones (UDL and DDL, respectively). The molecular weight of poly(ε-CL) of 25 000 was readily reached by ROP in toluene at 70 °C. It was reported that a covalently immobilized CALB, Amberzyme-CALB, and nanoPSG-CALB showed a high catalytic activity for the ROP of ε-CL.[207] Reaction parameters of enzymatic ROP of ε-CL with various lipases were investigated by conducting the reaction in toluene at various temperatures. Novozym 435 showed the highest catalytic activity at an optimal temperature of 65 °C in an optimal toluene/monomer ratio of 50/50. Polymerization and degradation during the reaction are competitive, and hence, the molecular weight versus reaction time relationship was an important factor. A new model to account for simultaneous polymerization and degradation including other factors was developed.[279] Multifunctional initiators from polyglycidols having primary OH groups were employed for initiating the ROP of ε-CL by enzymatic and chemical catalysts. Novzyme 435 and Zn(II) 2-ethylhexanoate were used for the enzymatic ROP and for the chemical catalyst, respectively. The obtained polymer archtectures were of core−shell polymers and the difference in structure was discussed for polymers produced via enzymatic and chemical processes.[280]

Oligomers from ε-CL gave poly(ε-CL) with M_n greater than 7.0×10^4, when the reaction was carried out at 70 °C in toluene.[266,268] Cyclic dimer of ε-CL (14-memberd) was polymerized by lipase CA, affording quantitatively poly(ε-CL) with M_n of 89 000 at a higher reaction temperature and also another large cyclic oligomer was polymerized.[268,281,282] Using a microbial polyester of low molecular weight telechelic hydroxylated poly[(*R*)-3-hydroxybutylate] (PHB-diol) as initiator, CALB-catalyzed ROP of ε-CL monomer was carried out in toluene or 1,4-dioxane mainly at 70 °C. PHB-diol has a primary and secondary OH groups at the terminal, and then, the polymerization of ε-CL was initiated selectively from the primary OH, giving rise to a diblock polyester with $M_n \approx 2400$ for PHB block and 1500 for ε-CL block. By variation of the content of two blocks and their molecular weight, T_g and T_m values of the block copolymers were tuned for thermoplastics.[283] In a similar direction, lipase CA-catalyzed ROP of cyclic oligomers prepared from 1,4-butanediol and dimethyl succinate gave a high molecular weight poly(butylene succinate) (PBS), with $M_w \approx 1.3 \times 10^5$, which was higher than that of the polyester obtained from direct polycondensation.[284]

Lipase-catalyzed ROP of ε-CL was conducted to coat the cellulose-fiber surfaces with hydrophobic poly(ε-CL) polyester chains, with utilizing the cellulose-binding module-CALB conjugate (CBM-CALB) as catalyst. The hydrophobicity of the resulted surface did not arise from the covalently attached poly(ε-CL) to the surface OH groups, but rather from surface-deposited polymers, which could be easily extracted.[285]

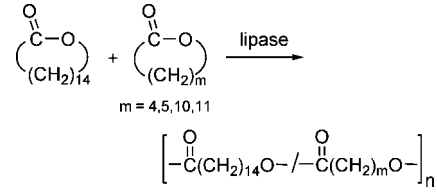
The lipase-catalyzed ROP reactivity of methyl substituted ε-CL monomers was examined, and it was revealed that ω-methyl ε-CL showed the least polymerizability among unsubstituted, α- and γ-substituted ε-CL monomers.[276] With the use of lipase CA, α-methyl ε-CL produced the corresponding polyester with M_n 11 400 ($M_w/M_n = 1.9$) in 74% yields in bulk at 60 °C for 24 h and α-methyl δ-VL afforded the polyester ($M_n = 8400$; $M_w/M_n = 2.0$) in 93% yields at 45 °C for 24 h.[277]

Cutinase from *Humicola insolens* (HiC) showed a high catalytic activity for ROP of ε-CL and PDL, for examples, the reaction of ε-CL with 0.1% w/w HiC at 70 °C in bulk for 24 h gave the polyester of $M_n = 16\,000$ ($M_w/M_n = 3.1$) in >99% yields, and monomer PDL with the same catalyst amount produced polyPDL of $M_n = 44\,600$ ($M_w/M_n = 1.7$) in toluene at 70 °C also in >99% yields.[217]

The microwave irradiation was applied on the Novozym 435-catalyzed ROP of ε-CL; in boiling toluene or benzene, a less-polar solvent, the polymerization rate was decelerated by the irradiation, whereas in boiling diethyl ether, a polar solvent, the rate was moderately accelerated to produce poly(ε-CL) with an increased M_n of 5800.[286]

A 4-membered lactone (β-propiolactone, β-PL) and substituted β-PLs were polymerized with lipase catalyst in bulk in 1996 by four groups, yielding linear polymers with molecular weight up to 2×10^4,[287−291] and also cyclic oligomers.[288] Propyl malolactonate (β-propyloxycarbonyl-β-PL) was polymerized with *Candida rugosa* lipase (lipase CR) catalyst via ROP in toluene and in bulk. The highest reaction rate was achieved in toluene with 2.11 M monomer at 60 °C at 10 wt % enzyme amount for the monomer, which was 25 times faster than the thermal polymerization observed. The maximum M_n value of 5000 of the polyester was obtained almost quantitatively. Thermal ROP of the polymer showed an M_n value of 1 800.[292]

Scheme 48



A 9-membered lactone (8-octanolide, OL) was also polymerized by lipase catalyst, producing the polymer with molecular weight of 1.6×10^4 at 75 °C after 10 days.[269,293] As to macrolides, 11-undecanolide (12-membered, UDL), 12-dodecanolide (13-membered, DDL), 15-pentadecanolide (16-membered, PDL), and 16-hexadecanolide (17-membered, HDL), were enzymatically polymerized.[259−262,294−298] At 75 °C for 10 days in bulk, polyUDL of $M_n = 23\,000$ ($M_w/M_n = 2.6$) was obtained quantitatively by lipase PF and the same polyester $M_n = 25\,000$ ($M_w/M_n = 2.2$) in 95% yields by lipase CC, which suggests a much higher ring-opening polymerizability of the macrolide than ε-CL.[269,293]

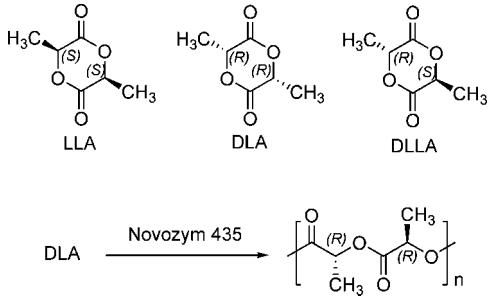
Ring-opening copolymerization of PDL with four monomers, δ-VL, ε-CL, UDL, and DDL, by lipase PF and lipase PC at 60 °C in bulk was carried out to give copolymers with M_n ranging 1200−6300, being not statistically random (Scheme 48).[259] ROP of PDL was investigated in more detail; at a low reaction water level, lipase I-PS-30 catalyzed polymerization of PDL at 70 °C gave polyPDL of M_n and M_w/M_n of 62 000 and 1.9, respectively.[297] Lipase CA (CALB) catalyzed the ring-opening copolymerization of PDL with 1,4-dioxan-2-one (DO) in toluene or diphenyl ether at 70 °C for 26 h gave a copolyester poly(PDL-*co*-DO) with high M_w (>30 000). A larger ring monomer PDL showed a higher polymerizability than a smaller counterpart DO.[299]

The largest lactone monomer having a simple unsubstituted structure so far studied is HDL (17-membered). ROP of HDL was performed by lipase CA, lipase CC, lipase PC, lipase PF, or PPL in bulk at 45−75 °C for 5 days, giving rise to polyHDL with M_n reaching to 5800 ($M_w/M_n = 2.0$) in good or quantitative yields.[298]

A 24-membered lactone derived from natural sophorolipid was polymerized via lipase-catalyzed ROP to give a glycolipid-based polyester. The reaction proceeded in two modes, depending on the reacted position of OH group with formation of monoacylated products to oligomers and polymers.[300] A 26-membered sophorolipid lactone having a double bond was prepared and subjected to ring-opening metathesis polymerization with a Ru catalyst, giving rise to poly(sophorolipids), with M_n exceeding 1.0×10^5.[301] Solid-state properties of the poly(sophorolipids) were examined in detail for glycolipid biomaterials.[302]

Lactide, a six-membered cyclic dimer of lactic acid, is a very important starting monomer for the production of poly(lactic acid) (PLA) as green plastics and other application materials. In 1997, lipase PS-catalyzed ROP of lactide was reported, where the ROP was carried out in bulk at a temperature between 80 and 130 °C to produce PLA with M_w up to 1.26×10^5, but the product yield was low. The DL-LA gave the higher molecular weight in comparison with LL-LA (LLA) and DD-LA (DLA) monomers.[303] Noticeably, a recent paper revealed that Novozym 435-catalyzed ROP of LLA did not take place, whereas that of DLA was enantioselectively induced to produce polyDLA of M_n value

Scheme 49



3300 (M_w/M_n = 1.2) in 33% yields. The polymerization employed the catalyst amount of 12.5% for DLA in toluene at a lower reaction temperature of 70 °C for 3 days (Scheme 49).[304]

Lipase-catalyzed ring-opening copolymerization of ε-CL with DL-lactide (DLLA) was carried out by CALB catalyst for the first time. Initially in the copolymerization, DLLA was consumed more rapidly than ε-CL, and the incorporation occurred as a LA dimer-wise. As the copolymerization proceeded, the relative amount of ε-CL increased. The nonrandom copolymer structure disappeared with time, probably because of a lipase-catalyzed transesterification reaction. During the copolymerization, macrocyclic compound formation was observed.[305] The similar copolymerization of the monomers DLLA, LLA, and glycolic acid (GA) by using lipase PC (*Pseudomonas cepacia*) catalyst and the copolymer's topology by using MALDI-TOF MS method were studied. The copolymerization between LA and glycolic acid (GA) (8:2 molar ratio) was conducted in bulk at 100 or 130 °C. Without the enzyme, copolymer was formed with a molecular weight of 1900, but the monomer conversion was low compared with that of the enzyme-catalyzed reaction. At 130 °C, a relatively high molecular weight polymer was produced after 7 days. The enzyme-catalyzed copolymerization produced a linear polyester with $M_w \approx 1.2 \times 10^4$ at 100 °C for 7 days, but a cyclic random copolymer with $M_w \approx 2.1 \times 10^4$ ($M_w/M_n = 4.0$) at 130 °C for 2 days, suggesting a possibility of complicated reactions.[306] For the enzymatic degradation and alkaline hydrolysis studies, various branched PLAs were synthesized via ROP of LLA, DLA, and DLLA using lipase PS catalyst at 140 °C with initiators of multifunctional alcohols like ethylene glycol, pentaerythritol, inositol, and polyglycerine.[307]

As observed generally in the polymerization reactions, the ring−chain (cyclic-linear structure) equilibrium exists also for enzymatic ROP[308] and enzymatic polycondensation (Scheme 36).[220] Lipase-catalyzed ROP of lactones accompanies the formation of cyclic oligomers in addition to major products of linear polyesters as noticed above for the ROP of ε-CL[177] and β-PL.[288] ROP of ε-CL was carried out using Novozym 435 as catalyst at 60 °C in bulk or in an organic solvent. In bulk, cyclic dimer of ε-CL and linear poly(ε-CL) were formed in 2% and in 98%, respectively. In acetonitrile the reaction gave a major portion of cyclic oligomers in total 70% (17% of dilactone and 53% of cyclic oligomers) and a minor portion of linear poly(ε-CL) in 30%. A similar tendency was observed also in THF and 1,4-dioxane, but in isooctane, the result was close to the bulk reaction (3 and 97%, respectively). Macrocycles up to 23 monomer units were produced in 1,4-dioxane.[308] The cyclic oligomer formation in the lipase-catalyzed ROP is explained by backbiting reaction of a polymer acyl−enzyme intermediate.

It is to be noted here that in addition to lipase catalysts, another hydrolase enzyme, the depolymerase, also catalyzes a polymerization reaction in vitro. A bacterial polyester of poly[(*R*)-3-hydroxybutylate] [P(3HB)] was degraded by the PHB depolymerase (EC 3.1.1.75), which is excreted as the extracellular enzyme, to produce monomeric and oligomeric 3-hydroxybutylates.[309] The first example to use the depolymerase as catalyst in vitro to form polyesters, a reverse reaction of the enzyme like in the catalysis of lipase, was reported for the enzymatic ROP of β-butyrolactone (β-BL, 4-membered; Scheme 54 $p = 1$).[310] PHB depolymerase is consisted of three domains; catalytic domain, linker region, and substrate-binding domain (SBD). Two types of PHB depolymerase from *Pseudomonas stutzeri* YM1006 were prepared; (A) one having all domains and (B) the other lacking SBD. ROP of β-BL was carried out in bulk using the catalyst (1−5 wt % for monomer) at 60−80 °C for 3 days. With catalyst A, the monomer conversion was up to 95% with M_w up to 1740, whereas with catalyst B, the corresponding values were 97% and 3420, respectively. Catalyst B showed rather higher activity, indicating that SBD was not necessary for the in vitro polymerization.

Catalytic activity in ROP of *(R)*-β-BL catalyzed by the PHB depolymerase was examined for four catalysts; a wild-type from *Alcaligenes faecalis* T1 and three kinds of site-specific mutants of a catalytic triad (Ser-His-Asp) of the depolymerase, S139A, D214G, and H273D (S = Ser, A = Ala, D = Asp, G = Gly, and H = His). Among these four, only the wild-type PHB depolymerase showed the ROP activity; at 80 °C in bulk with the catalyst amount of 3 wt % toward the monomer for 24 h, yielding PHB with M_w = 5000 in 93% conversion of monomer. All the mutants were virtually inactive. The results demonstrated the importance of the catalytic triad amino acid residues, in particular the serine residue, which is common in the active site, in a lipase box pentapeptide Gly-X-Ser-X-Gly, to serine hydrolases such as lipase, esterase, and serine protease.[311]

In the enzymatic ROP of various lactones catalyzed by the PHB depolymerase from *Alcaligenes faecalis* T1, correlation of lactone structure and catalytic activity was examined.[312] Among the unsubstituted lactones (4-, 5-, 6-, 7-, 12-, and 13-membered; Scheme 47), 4- and 6-membered lactones (β-PL and δ-VL, respectively) showed the highest polymerization reactivity. β-PL gave the polyester with M_w = 16 400 (M_n = 10 800) in 98% monomer conversion, and in the δ-VL case M_w = 2900 (M_n = 2000) in 48% monomer conversion. On the other hand, ε-CL (7-membered) showed little polymerizability, and macrolides did not show the reactivity. The results are interestingly compared with those of the lipase catalysis, in which larger-ring-sized monomers tend to show a higher reactivity. This was explained by a smaller size of the active site cleft of the depolymerase. In addition, ROP of (*R*,*S*)-β-BL by the depolymerase resulted in the enantioselection to give R-enriched optically active PHB.

The PHB depolymerase from *Pseudomonas lemoignei* was used for esterification of lactones (ε-CL, δ-BL, and γ-BL) and lactide, and also for ROP of ε-CL and trimethylene carbonate in organic solvents like benzene, cyclohexane, and acetonitrile.[313]

4.2.1.2. Reaction Solvents. Lipase-catalyzed ROP is normally carried out in bulk or in an organic solvent, such as toluene, heptane, 1,4-dioxane, diisopropyl ether, and dibutyl ether. In addition, water, supercritical carbon dioxide (sc-

CO_2), and ionic liquids, which are regarded as typical "green solvents", could be employed as solvent.

The first example using a water solvent used five lactone monomers, ε-CL, OL, UDL, DDL, and PDL, for the lipase-catalyzed ROP (Scheme 47). Macrolides of UDL, DDL, and PDL were polymerized by lipase in water to produce the corresponding polyesters up to 89% yields. *Pseudomonas cepacia* (lipase PC) showed the best results in terms of polyester yields and molecular weight.[261,314] Typically, a mixture of UDL (2 mmol) and lipase PC (100 mg) in water (10 mL) was stirred at 60 °C for 72 h, giving rise to polyUDL with $M_n = 1300$ ($M_w/M_n = 2.1$) in 79% yields. DDL is hardly soluble in water; however, the addition of the lipase gave a white emulsion-like solution, which allowed the ROP. In contrast, a mixture of the lipase and ε-CL or OL did not induce the ROP, which failed to form an emulsion-like system.

The second example of the water medium system is the lipase-catalyzed ROP of a lactone in miniemulsions.[315] A typical reaction is given; a mixture of PDL monomer (2.4 g, 10 mmol), water (10 mL, containing 1.0 wt % Lutensol AT50; nonionic surfactant having a PEG chain of molecular weight 2 000), and hexadecane (100 mg) was vigorously stirred for 1 h at 45 °C to give miniemulsions. To the mixture, a suspension of lipase PS (50 mg) in 5.0 g of the surfactant solution was added, and the resulting miniemulsions, consisting of PDL nanodroplets, were subjected to ROP with stirring at 45 or 60 °C up to 24 h to reach a full conversion of PDL. PolyPDL nanoparticles were thus obtained, which is considered to be a direct synthesis of biodegradable polymer nanoparticle (size <100 nm). PolyPDL showed a bimodal molecular weight distribution; the majority was that of high molelcular weight ($>2.0 \times 10^5$). In the presence of an unsaturated alcohol or acid, such as linoleic acid, they were introduced into the polymer terminal via extensive esterifications to give a functionalized polyPDL with a polymerizable group.

A further paper described a lipase-catalyzed ROP of lactones catalyzed by Novozym 435 encapsulated in either the water pool or the bilayer of polystyrene-polyisocyanopeptide block copolymer, which forms stable polymersomes with a bilayer structure.[316] Four lactones, ε-CL, OL, DDL, and a cyclic diester 1,4,7,10-tetraoxacyclotetradecane-11,14-one, were used for varying the nature of monomers. The lipase was located both in the water pool and in the bilayer and catalyzed the ROP of the lactones. The catalytic activity of the lipase was similarly observed for OL and DDL in the polymersome water pool similarly as in a free water system; polyOL up to molecular weight of ~3000 and polyDDL up to ~1200 were produced. But, in the bilayer, the product oligomers were shorter probably because the monomer is less sterically accessible to the catalyst.

Supercritical carbon dioxide ($scCO_2$) is inexpensive, inert, nontoxic, and nonflammable, and possesses potentials for polymer synthesis and recycling. $scCO_2$ was used for the first time for the lipase CA-catalyzed ROP of ε-CL to produce poly(ε-CL) with molecular weight $M_w \approx 1.1 \times 10^4$ in high yields and for the ring-opening copolymerization between ε-CL and DDL to produce poly(ε-CL-*co*-DDL) with $M_w \approx 1.3 \times 10^4$.[239] A further work reported the synthesis of poly(ε-CL) having a higher molecular weight (M_w) reaching 7.4×10^4. The enzyme and $scCO_2$ were repeatedly used for the polymerization.[317] The kinetics of CALB-catalyzed ROP of ε-CL in $scCO_2$ at 35 °C was investigated using a high-pressure sampling autoclave. The polymerization was approximately first order with respect to monomer up to 80% conversion. Effects of water-content in $scCO_2$ on the reaction rate and molecular weight were examined. The product molecular weight was high up to 5.0×10^4, when the reaction system was dry. The molecular weight control was poor, which was attributed to the large degree of transesterification, forming both linear polymers (intermolecular transesterification) and cyclic compounds (intramolecular transesterification). This behavior has been observed also in conventional solvents.[318] The lipase-catalyzed degradation of poly(ε-CL) in the presence of acetone produced oligomers with molecular weight less than 500 in $scCO_2$.[319,320] The produced ε-CL oligomer was again polymerized with lipase CA to yield poly(ε-CL) having M_n greater than 8.0×10^4.[320] A chemoenzymatic method combining the enzymatic ROP and atom-transfer radical polymerization (ATRP) techniques used $scCO_2$ as solvent for the synthesis of block copolymers containing ε-CL and MMA.[321−323]

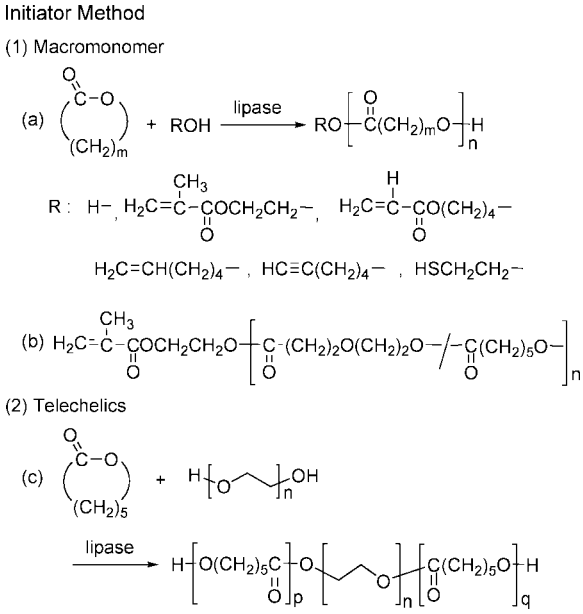
Ionic liquids are nonvolatile, thermally stable, and highly polar liquids, which allow dissolution of many organic, inorganic, and metallo-organic compounds, as well as polymeric materials. Thus, ionic liquids become popular for the synthesis and modification of polymers from the standpoint of green character.[324] Lipase-catalyzed ROP of ε-CL was realized for the first time in an ionic liquid solvent, such as 1-butyl-3-methyl-imidazolium salts ([bmim][X^-]), giving rise to poly(ε-CL) with $M_n = 4200$ ($M_w/M_n = 2.7$) in 97% yields at 60 °C.[204] Novozym 435-ctalyzed ROP of ε-CL in three ionic liquids, [bmim][BF_4], [bmim][PF_6], and [bmim]-[$(CF_3SO_2)_2N$], at 60 °C for 24 h produced poly(ε-CL) of a higher M_n value of 7000−9500 (M_w/M_n ~2.4) in good yields.[227]

With using the same ionic liquid, ROP of lactide (LA) by lipase CA catalyst at 130 °C produced polyLA having molecular weight values of ~36 000 and ~5000, where water content in the solvent was varied from 0 to 5%.[325]

4.2.1.3. End-Functionalized Polyesters. Synthesis of end-functionalized polymers, typically macromonomers and telechelics, is both fundamentally and practically important in polymer chemistry, which requires a precise control of the polymer terminal structure. A number of methodology for synthesis of such polymers have been developed; however, they often needed tedious and time-consuming procedures. Lipase catalysis, however, provides a novel method for a single-step synthesis of end-functionalized polyesters via a relatively simple reaction. Moreover, enzyme-catalyzed synthesis gives product polymers free from any metal catalysts, that broadens the application scope of the products in particular for biomedical applications.

Initiator Method: A speculated mechanism of lipase catalysis involves a nucleophile like water and an alcohol to initiate the ROP of lactones. In fact, lipase CA-catalyzed ROP of ε-CL or DDL at 60 °C in bulk in the presence of a functional alcohol produced end-functionalized polyesters ("initiator method").[326] Functional alcohols include 2-hydroxyethyl methacrylate (HEMA), 5-hexen-1-ol and 5-hexyn-1-ol for the synthesis of methacryl-, ω-alkenyl- and alkynyl-type polyester macromonomers having M_n 1 000−3 100. The functionality reached 100% in many reaction runs (Scheme 50a). In order to synthesize comb polymers using a macromonomer technique, macromonomers with various molecular weight were prepared via lipase-catalyzed ROP of ε-CL and 1,5-dioxepane-2-one (DXO) with the use of HEMA

Scheme 50



as initiator.[327] The synthesis was carried out at 60 °C in bulk with lipase CA catalyst by varying the monomer to initiator feed ratio from 40 to 250 to give (meth)acryl-type macromonomers with M_n = 7100−20 800. The amphiphilic nature of macromonomers was controlled with using poly(ε-CL) for hydrophobic chain and polyDXO for hydrophilic chain, and thus an amphiphilic poly(ε-CL-*co*-DXO) macromonomer was obtained (Scheme 50b). Likewise, the lipase CA-catalyzed ROP of ε-CL or DXO initiated by 4-pentene-2-ol gave a macromonomer having a double bond. As macroinitiators, poly(ethylene glycol) and poly(ε-CL)-diol initiated the ROP of ε-CL or DXO to give a triblock polyester with a hydroxy group at both ends (Scheme 50c).[328] The methacryl-type polyester macromonomer was derived via lipase-catalyzed ROP of PDL initiated with HEMA and radically polymerized to lead to polymers of a brush structure.[329]

A primary alcohol group in the 6-position of alkyl glucopyranosides[330,331] and six primary alcohol groups of a first generation dendrimer[332] induced the ROP of ε-CL with lipase CA catalyst, where the regioselective initiation (monoacylation of the initiator) took place. Thus, the primary hydroxy group was regioselectively acylated to give the polymer bearing the sugar moiety at the polymer terminal.

The initiation process was examined under various reaction conditions at 60 °C in bulk or in toluene, which relates to synthesize end-functionalized polymers by introducing the functionality into polymers via ROP (initiator method).[333] In the lipase-catalyzed ROP of lactones, an initiator nucleophile species can be water, an alcohol, an amine, or a thiol. Initiation reaction behaviors in the Novozym 435-catalyzed ROP of ε-CL using water, benzyl alcohol, and other Br-containing primary alcohols as initiators were investigated. Among these nucleophiles, water was most reactive and hence a good "initiator". To obtain highly functionalized polyesters the enzyme and the reaction system were to be dried to reduce water content. However, it is also true at the same time that a completely dry reaction system could not be realized because the enzyme requires traces of water to retain its activity.

When the lipase CA-catalyzed ROP of ε-CL or PDL in the presence of HEMA was carried out at a higher temperature of 80 °C in bulk, the reaction became very complicated.[334] Not only the HEMA-initiated ROP but also transesterification among product polymers, monomer, and HEMA took place extensively after a longer reaction time. Thus, these reactions resulted in the expected product of HEMA-end-capped macromonomer, as well as various transesterification products because of methacrylate transfer and polyester transfer, containing polyesters with four different end-group structures; HEMA-end group, hydroxy-end group, 1,2-ethanediol-end group, and mathacrylated hydroxy-end group. By utilizing the transesterification, dimethacrylated polyesters could be readily obtained in one-pot reaction. It was suggested that for the selective preparation of the desired macromonomer, ROP is to be carried out under mild conditions and terminated at a lowed monomer conversion. Further, the transesterification behaviors were compared between cases of HEMA initiator and 2-hydroxyethyl acrylate (HEA) initiator.[335] In both cases, transesterification occurred extensively at 80 °C in bulk, and the reaction rate was 15 times faster on the HEA-initiated polyesters. It was suggested that transesterification reactions have to be always considered when perfoming an enzyme-catalyzed ROP.

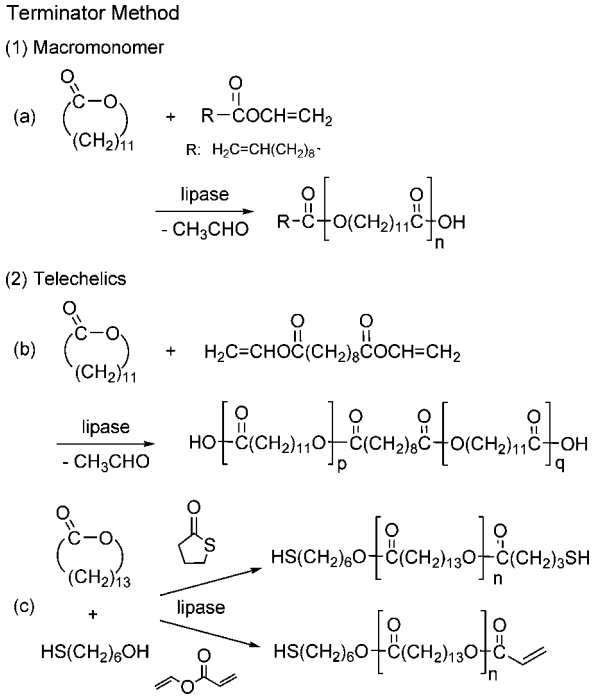
Thiol end-functionalization was achieved by CALB catalyst to induce the ROP of ε-CL with 2-mercaptoethanol initiator. The fraction of thiol functionalized-ends of poly(ε-CL) was 70% with M_n = 3100 (Scheme 50a).[336]

Enzymatic ROP was applied to synthesize hyperbranched aliphatic copolyesters by copolymerization of ε-CL with 2,2-bis(hydroxymethyl)butylic acid, catalyzed by lipase CA. In preparing the AB_2 polyesters, the degree of branching and density of functional end group were controlled.[337] A new approach to a biodegradable polyester system was performed by the lipase-catalyzed ROP of ε-CL and 1,4-dioxan-2-one (DO) monomers initiated from an alcohol attached on the gold surface. The polyester system can be used as a biocompatible/biodegradable polymer for coating materials in biomedical area such as passive or active coatings of stents. This method would be beneficial in the applications where the minimization of harmful species is critical.[338] A similar surface-initiated polymerization was reported for the in situ solid-phase synthesis of biocompatible poly(3-hydroxybutyrate).[195] A macroinitiator of a linear or a four arm star-shaped polyglycidol was used for synthesis of densely grafted poly(glycidol-*graft*-ε-CL) and loosely grafted poly[(glycidol-*graft*-ε-CL)-*co*-glycidol] copolymers via lipase-catalyzed ROP of ε-CL or Sn-catalyzed chemical process using ε-CL monomer. Architecture, microstructures, molecular weight, and chemical compositions of the copolymers were examined and compared. In comparison with linear poly(ε-CL), the latter copolymer showed a change of degradation mechanism and much enhanced degradability, probably because of the high concentration of hydroxy groups at the polyglycidol backbone.[339]

The initiator method is useful for a single-step synthesis of end-functionalized polyesters as well as other polymers, which can be prepared via polymerization of a monomer induced by a lipase/functional alcohol (or another nucleophile) catalyst system. The polymerization, however, is to be carried out under the conditions to minimize transesterifications for producing precisely functionalized polyesters.

Terminator Method: A single-step, convenient production of end-functionalized polyesters was developed by lipase-catalyzed ROP of DDL in the presence of vinyl esters (vide

Scheme 51



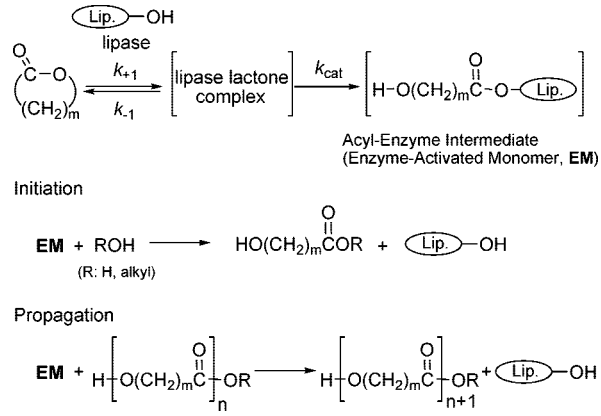
infra).[162,340] The vinyl ester acted as terminator during the polymerization (terminator method). In use of vinyl (meth)acrylate as terminator, the (meth)acryl group was quantitatively introduced at the polymer terminal at 60 °C in bulk to give a (meth)acryl-type polyester macromonomer; with M_n = 2000−4000 and functionality >0.95. The polymerization in the presence of vinyl 10-undecanoate produced the ω-alkenyl-type macromonomer (Scheme 51a). This system was applied to the synthesis of telechelics having a carboxylic acid group at both ends by using divinyl sebacate in the reaction mixture at 60 °C in bulk; the M_n value of the telechelics was 2900 with functionality of 1.95 (Scheme 51b).[341]

An enzymetic one-pot synthesis gave difunctional telechelics. The CALB-catalyzed ROP of PDL was conducted at 60 °C by 6-mercaptohexanol initiator and then terminated with γ-thiobutyrolactone to give a telechelic polyester with very high content of thiol−thiol end-groups and with vinyl acrylate to produce that of thiol−acrylate end-groups (Scheme 51c).[342] A telechelics having thiol−thiol end-groups of polyPDL was synthesized by lipase-catalyzed ROP of PDL monomer with using 6-mercapto-1-haxanol as initiator and γ-thiobutyrolactone as terminator and then used further for the preparation of semicrystalline polymer networks.[343]

Lipase-catalyzed ROP of ε-CL was extended for the synthesis of amphiphilic macromonomers containing poly(ε-CL); first, ROP of ε-CL was initiated by lipase catalysis from OH containing initiators like a hexahydroxy dendrimer and ethyl glucopyranoside, and then, (meth)acryloyl polymerizable group was introduced to terminal OH of ε-CL chain by end-capping with vinyl (meth)acrylate terminator (Scheme 51a).[344−346]

The terminator method involves a lipase-catalyzed single-step acylation of a polyester alcohol end-group with a vinyl ester or a lactone, the process of which can be applied basically for other polymer alcohols to give an end-functionalized polymer.

Scheme 52



4.2.1.4. Mechanism and Monomer Reactivity of Ring-Opening Polymerization. A mechanism of lipase catalyzed ROP of lactones is postulated by considering the principal reaction course involving an acyl-enzyme intermediate (Scheme 52).[8,17,18,25,264,294] Lipase-catalyzed hydrolysis of an ester in vivo is generally accepted to proceed via a similar acyl−enzyme intermediate.[347] The catalytic site of lipase is known to be $-CH_2OH$ group of a serine-residue. The key step is the reaction of lactone with lipase catalyst involving an enzyme−lactone complex and ring-opening of lactone to give an acyl-enzyme intermediate (enzyme-activated monomer, EM). The initiation is nucleophilic attack of a nucleophile such as water or an alcohol, which is present in the reaction mixture, at the acyl carbon of the intermediate to produce ω-hydroxycarboxylic acid (n = 1); the shortest propagating species. In the propagation stage, EM is nucleophilically attacked by the terminal hydroxy group of a propagating chain end to produce a one-monomer-unit elongated polymer chain. Thus, the polymerization proceeds via an "activated monomer mechanism". This mechanism is different from an "active chain-end mechanism" observed normally for many other polymerizations, typically a vinyl polymerization, where a propagating chain-end is active, reacting with a monomer to give a one-more-monomer unit elongated, propagating polymer chain.

As to the aspect of ROP reactivity of the monomers, Table 2 shows dipole moment values and reaction rate values of five kinds of reaction of unsubstituted lactones with different ring size. Many accumulated data show the ring-opening reactivity is governed primarily by the ring strain. The dipole moment value of the monomers is indicative of polarity of the molecule and is taken as a measure of their ring strain. The value of macrolides is lower than that of ε-CL and close to that of an acyclic fatty acid ester (butyl caproate), suggesting a very small ring strain of the macrolides. In fact, the rate constants of the macrolides in alkaline hydrolysis and anionic polymerization with NaOMe initiator are much smaller than those of ε-CL, although the hydrolysis took a little peculiar behaviors for medium sized lactones, paticularly NL and DL. These data generally imply that the macrolides have much lower ring strain, and hence, show less reactivity and polymerizability than ε-CL with an anionic catalyst.

Early studies qualitatively indicated that macrolides, such as UDL, DDL, and PDL, showed higher reactivity than smaller ring lactones like ε-CL. The macrolides gave the corresponding polyesters with higher molecular weight in higher yields than ε-CL.[259,294−296] In 1997, the first kinetic

Table 2. Comparison in Dipole Moment Values and Reaction Rate Values of Unsubstituted Lactones with Different Ring Size

		rate constant		relative rate of polymerization		
				enzymatic polymerization		
lactone (ring size)	dipole moment (C·m)	alkaline hydrolysis[a] ($L \cdot mol^{-1} \cdot s^{-1}$, $\times 10^4$)	propagation[b] (s^{-1}, $\times 10^3$)	A[c]	B[d]	Zn-catalyzed polymerization[e]
δ-VL (6)	4.22	55 000		0.10 (0.14)	0.07	2,500 (2,800)
ε-CL (7)	4.45	2550	120	0.10 (0.14)	0.15	330 (370)
HL (8)	3.70	3530			3.8	
OL (9)	2.25	116			0.45	21 (23)
NL (10)	2.01	0.22			0.04	
DL (11)	1.88	0.53			0.02	
UDL (12)	1.86	3.3	2.2	0.13 (0.18)	0.06	0.9 (1.0)
DDL (13)	1.86	6.0	15	0.19 (0.26)	0.37	1.0 (1.1)
PDL (16)	1.86	6.5		0.74 (1.0)	1.0	0.9 (1.0)
HDL (17)				1.0 (1.35)		1.0 (1.1)
butyl caproate	1.75	8.4				

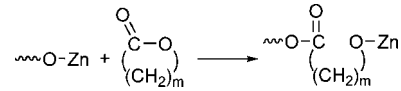
[a] With alkaline of NaOH in 1,4-dioxane/water at 0 °C.[348] [b] NaOMe initiator in THF at 0 °C.[349] [c] Lipase PF as catalyst in *i*-propyl ether at 60 °C; the relative rate is given by normalizing the V_{max}/K_m values with respect to HDL and to PDL in the parentheses.[350] [d] Novozym 435 as catalyst in toluene at 45 °C; the relative rate is given by calculating the V_{max}/K_m values from Michaelis−Menten kinetics parameters and by normalizing the values with respect to PDL.[263] [e] $Zn(Oct)_2$ initiator in bulk polymerization at 100 °C; the relative rate is given by normalizing the initial rate constants with respect to HDL and to PDL in the parentheses.[351]

analysis based on the Michaelis−Menten equation was reported for the lipase PF (*Pseudomonas fluorescens* lipase)-catalyzed ROP of ε-CL and DDL.[260] The lipase-catalyzed ROP obeyed the Michaelis−Menten kinetics. The formation of an acyl-enzyme intermediate (EM in Scheme 52) was considered to be the rate-determining of the overall reaction because the reactivity of the intermediate must be high and hence the subsequent steps are rapid. This hypothesis was supported by the experiments using a different amount of nucleophiles, water and 1-octanonl, in the polymerization reaction. The Michaelis−Menten kinetics parameters, K_m and V_{max} values, were determined and it was concluded quantitatively that DDL is 1.9 times more reactive than ε-CL in ROP.[260,261]

The V_{max}/K_m (s^{-1}) value is a good measure for the overall rate of the enzymatic ROP. Thus, the relative rate of lactone monomers with different ring size was derived by normalizing the values with respect to a specific lactone monomer (Table 2).[259−261,263,350−353] There is a tendency that with lipase PF the larger the ring-size, the larger the polymerization rate in one magnitude difference as seen in column A, that is, the higher polymerizability of the macrolides was explained by the higher rate in the formation of EM. As for the Michaelis−Menten constant K_m ($(k_{-1} + k_{cat})/k_{+1}$, $mol \cdot L^{-1}$), the values are not much different each other; they are from 0.61 to 1.1, on the other hand, the maximal reaction rate V_{max} (k_{cat} $[E]_0$, $mol \cdot L^{-1} \cdot s^{-1}$) values are much more varied from 0.66 to 7.2.[350] These results suggested that the reaction rate is mainly governed by the larger value of V_{max} and much less by the binding ability. In other words, the reaction process from the lipase−lactone complex to form the intermediate EM is the key step in the lipase-catalyzed ROP (see Scheme 52).[257]

On the other hand, the Zn-catalyzed anionic polymerizability of lactones showed the reverse direction with big difference, for example, δ-VL and ε-CL showed 2500 and 330 times more reactive than the macrolides (Table 2). The polymerizability is mainly governed by the ring-strain. In the propagation reaction, the rate-determining is the nucleophilic attack of the propagating Zn-alkoxide species at the carbonyl carbon followed by the scission of the acyl-oxgen bond and the reformation of the alkoxide species (Scheme 53).[351]

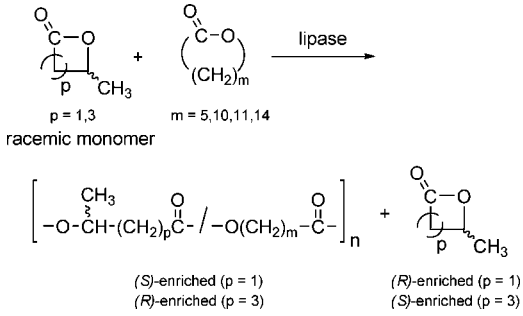
Scheme 53



Nowadays, lipase CA (Novozym 435) is widely used; its structure was determined by X-ray crystallographic analysis.[354] In comparison with lipase PF, catalysis of lipase CA was examined and found much different in behaviors. Lipase CA exhibited a higher catalytic activity for ε-CL than for macrolides. Qualitatively, initial rates ($\times 10^5$, $L \cdot mol^{-1} \cdot h^{-1} \cdot mg^{-1}$) of ROP by lipase CA catalyst were obtained as 2 300 for ε-CL, 48 for OL, 1 600 for DDL, and 4 700 for PDL. For these four monomers, the corresponding values of lipase PF were 1.3, 1.8, 2.5, and 8.5, and those of lipase PC were 0.42, 2.2, 4.8, and 11.[269,278,293]

Catalysis of lipase CA for ROP of nine lactones was investigated according to Michaelis−Menten kinetics.[263] The values of K_m are in a narrow range between 0.09 and 0.73 $mol \cdot L^{-1}$, suggesting close affinities of the lipase for all lactones. On the other hand, the values of V_{max} varied between 0.07 and 6.10 $mol \cdot L^{-1} \cdot h^{-1}$ and did not show a trend. These are partially similar to the results observed above for lipase PF catalysis,[261,262,350] indicating that the ROP reactivity with lipase CA catalyst is likely operated also by the process from the lipase−lactone complex to form EM in Scheme 52. The relative rate of ROP of nine monomers was derived from V_{max}/K_m values (column B in Table 2). The most reactive monomer HL (8-membered) showed almost 200 times more reactive than the least reactive DL (11-membered). The relative rate values look very complicated; there is no monotonous change depending on the ring size. Medium sized lactones showed rather smaller reactivity. These results cannot be related to variations in physical properties such as the dipole moment of lactones only. Simple explanations are not available, but in addition to the dipole moment value and the ring strain, other factors, such as the transoid and cisoid structure of lactones[348] and the conformational strain and transannular interactions in medium ring lactones, were taken into account for the respective ring size cases.[263] Immobilized *Humicola insolens* cutinase (HIC) was used as catalyst for kinetics and mechanistic studies on ROP of ε-CL and PDL in bulk at temperature range from 50 to 90 °C. The results revealed that the

Scheme 54



propagation rate was first order with respect to monomer and consistent with a chain-end propagation mechanism, without chain termination. The turnover number (TON, mol of ε-CL converted to polymer per mol of cutinase per min) was determined as 770, a maximum value, that was obtained at 70 °C. Catalytic activity of HIC for both monomers was close to that of Novozym 435 in terms of the polymer molecular weight.[355]

A different approach of copolymerization method gave some new insight into the ROP mechanism. Lipase CA-catalyzed copolymerization using two ω-methyl substituted lactones (racemic) and four achiral lactones (unsubstituted) was investigated.[352] The copolymerization of β-butyrolactone (β-BL, 4-membered) with an achiral lactone at 60 °C induced enantioselection of the racemic monomer, where *(S)*-β-BL was preferentially consumed to give *(S)*-enriched copolyester (Scheme 54, $p = 1$). In the propagation, there are four different elemental reactions, which are given as A, B, C, and D in Figure 13, showing the steps where an acyl-enzyme intermediate (or transition-state) is attacked by a nucleophile of propagating end of hydroxy group.[352,353] From the ^{13}C NMR analysis of the four diad peaks, the intensity ratio was

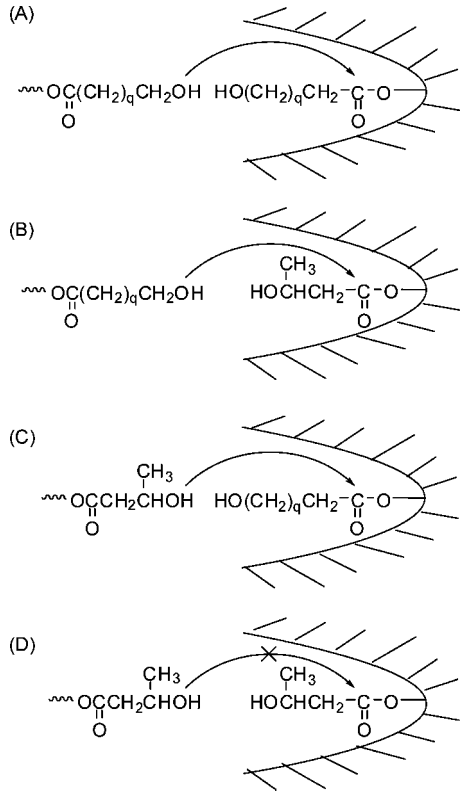


Figure 13. Four elemental propagation steps in the copolymerization of β-BL with an unsubstituted lactone.

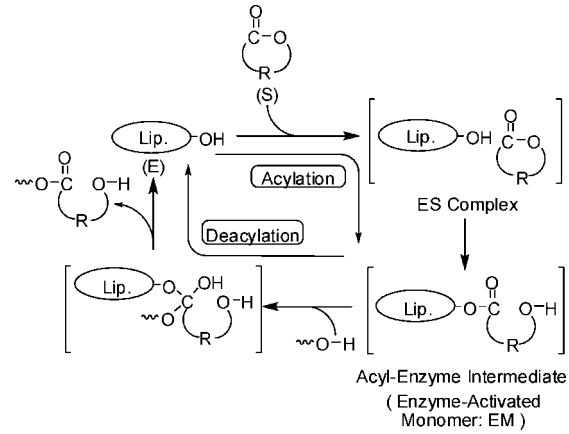


Figure 14. A general illustrative mechanism of lipase-catalyzed ROP of lactones. (Reprinted with permission from ref 25. Copyright 2009 Wiley-VCH.)

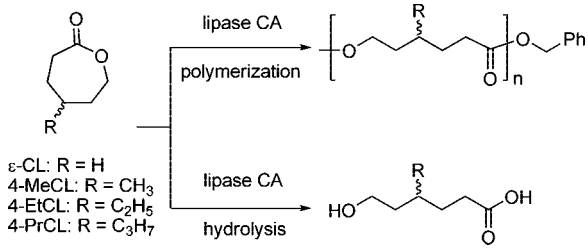
approximately A/B/C/D = 10:6:4:0, respectively. If the rate-determining step of the lipase-catalyzed ROP is the formation of EM because of a high reactivity of EM, the formation of diads A and C, and of diads B and D should be the same in amount; however, the results were different; they are 10:4 and 6:0, respectively. This suggests that the reaction steps of Figure 13 involve a propagating chain end of sterically two different nucleophile structures (primary and secondary alcohols), and hence, the structure of the nucleophile greatly affects the overall polymerization.

The lipase CA-catalyzed copolymerization of racemic δ-CL (4-methyl-VL, 6-membered) with DDL proceeded in a similar way; however, the product copolymers were all enriched in *(R)*-isomer of δ-CL, in a reverse enantioselection to β-BL (Scheme 54, $p = 3$). The peaks intensity corresponding to A, B, C, and D of the δ-CL reaction was in about 10:3:3:0.5, that is a similar trend to the above copolymerization of β-BL with DDL.[352] Similar views can be made again as to the lipase-catalyzed ROP mechanism.[353]

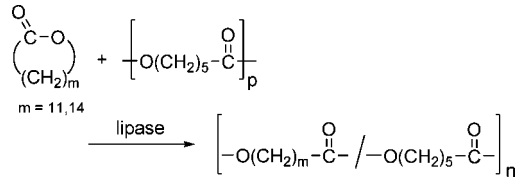
From these observations, the rate-determining step is not always the formation of the acyl-enzyme intermediate (EM) in Scheme 52. It seems more general to consider a mechanism of the lipase catalyzed ROP, in which the formation of EM (acylation of lipase) or the subsequent reaction of EM with monomer (deacylation of lipase) are operative depending on the monomer structure. In particular, the deacylation step becomes more important when the propagating alcohol end is of sterically bulky nucleophile, and then the step determines the overall rate of reaction. Enantioselection is therefore induced possibly at both acylation and deacylation steps (Figure 14).[347,353]

The above view was further supported by the lipase PF-catalyzed copolymerization, giving the structure of propagating chain-end with both a primary alcohol like a case between unsubstituted lactones, PDL and ε-CL. Four diad sequence ratios of the product copolyester were roughly in the following values: PDL − *PDL* = 10, PDL − *ε-CL* = 2.4, ε-CL − *PDL* = 9.6, ε-CL − *ε-CL* = 2.7, where the italics denote the monomer of EM. The homopolymerizability ratio of PDL to ε-CL is 10/2.7 = 3.7, which can be compared with the value of 7.4 obtained from each homopolymerization in Table 2. The competitive reaction rate ratio of PDL and ε-CL chain-ends toward EM of PDL is 10/9.6 = 1.04, which is almost the same, and that of PDL and ε-CL chain-ends toward EM of ε-CL is 2.4/2.7 = 0.89, which is also very close. These results imply that the EM mainly governs the

Scheme 55



Scheme 56

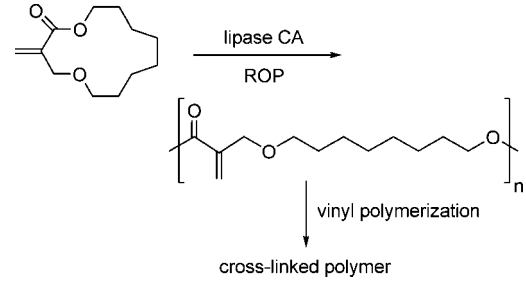


overall reactivity of the monomer, regardless of the kind of the propagating chain-end so far as its structure is similar (a primary alcohol in all cases), that is, the ROP mechanism involving the formation of the EM complex as a rate-determining step is plausible.[259] Similar cases were reported in the lipase PF-catalyzed copolymerizations between δ-VL and ε-CL (unsubstituted 6- and 7-membered, respectively),[253] and between OL and ε-CL or DDL,[293] to give a random copolyester, where homopolymerizabilty and cross-reactivity are close each other for these monomers.

A mechanism illustrating the importance of the deacylating step was previously described, from the results of lipase CA catalyzed ROP of 4-alkyl-substituted ε-CLs where methyl, ethyl, or propyl group was employed as an alkyl group (Scheme 55).[356] The following was observed: (i) the polymerization rate was much affected by the substituent size in the relative values of $H/CH_3/C_2H_5/C_3H_7 = 140:70:14:1$ and (ii) the enantioselectivity changed from (*S*) for CH_3 and C_2H_5 to (*R*) for C_3H_7, with greatly decreasing upon increasing the substituent size. On the other hand, in the hydrolysis of these monomers by the same enzyme, (i) the relative rate was for $H/CH_3/C_2H_5/C_3H_7 = 2.5:5.8:5.2:1$, and (ii) the enantioselectivity was (*S*) in all cases. From these observations, it was suggested that the deacylation step is more likely to be the rate-determining for the polymerization; the reaction of nucleophile alcohols having different structures (with bulky or less bulky alkyl) with EM caused the polymerization rate difference and the shift of enantioselection. However, in the hydrolysis, the rate-determining step is most likely the formation of the acyl-enzyme intermediate, because water is a very small nucleophile while a propagating chain end alcohol is not.

In addition to the main reaction modes in Scheme 52 and Figure 14, a transesterification often takes place under severe reaction conditions during the lipase-catalyzed ROP of lactones. For example, when the ROP of a macroride (DDL or PDL) was carried out in the presence of an aliphatic polyester (poly(ε-CL)) or poly(1,4-butylene adipate)), a polyester copolymer from the cyclic monomer and the polyester was produced (Scheme 56).[357,358] Also, lipase CA- or lipase PF-catalyzed intermolecular transesterification between two different polyesters took place to give a polyester copolymer composed from the two polyester repeating units (Scheme 44).[247,357,358] Copolymerization of ε-CL with DL-lactide was carried out with lipase CA catalyst and the product copolyester was structurally well characterized.[305]

Scheme 57



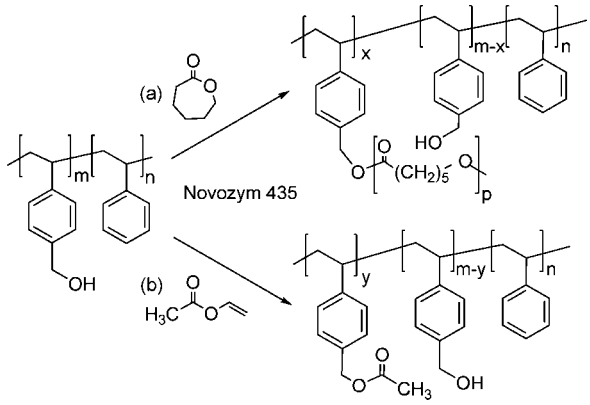
4.2.1.5. Chemoselctive and Regioselective Polymerizations. The lipase catalyst chemoselectively induced the ROP of 2-methylene-4-oxa-12-dodecanolide, a cyclic derivative of methyl methacrylate, yielding a polyester having the reactive exo-methylene group in the main chain (Scheme 57). This type of polymer is hardly obtained by using a conventional chemical initiator. The chemoselective polymerization of α-methylenemacrolides having various groups in the ring, for example, aromatic, ether, or amine group, was enzymatically, anionically, and radically carried out. The lipase-catalyzed polymerization selectively afforded polyesters through the ring-opening process, whereas anionic and radical initiators induced the vinyl polymerization. The polyester was readily cross-linked radically at 60 °C in toluene in 15 min via reaction through the reactive methacrylic methylene group to produce a cross-linked polymer.[359,360]

A chemoselective ROP of Ambrettolide (Am) epoxide, a 17-membered macrolide having an epoxy group at 10-position (Figure 12), was performed by Novozym 435 catalyst to afford the polyester with M_n value of 9700 ($M_w/M_n = 1.9$). The epoxide group remained unaffected during the polymerization.[179] Related functional macrolides, Globalide (Gl) and Am, are presently used in fragrance industry. Gl is a 16-membered lactone having the double bond at 11 or 12 position, and Am is a 17-membered lactone with the double bond at 10 position. They can be compared with simple macrolides of PDL and HDL, respectively. Novozym 435-catalyzed ROP of Gl and Am were performed in toluene at 60 °C in the presence of molecular sieves to give polyGl and polyAm, having M_n both around 2.4×10^4. Both polyesters had melting points 46−55 °C, which are compared with those of saturated polyesters, polyPDL and polyHDL, of around 95 °C. PolyGl and polyAm were nontoxic, hydrolytically and enzymatically nondegradable biomaterials. The unsaturated polyesters were radically cross-linked at 170 °C to give transparent films, in which the endo-double bond was involved in the reaction.[361]

Regioselective ROP of ε-CL was initiated from a sugar molecule with lipase-catalyst. The primary hydroxy group in the 6-position of methyl or ethyl glucopyranoside induced the ROP of ε-CL in a high conversion of the monomer at 70 °C. Lipase CA and PPL showed a high catalytic activity.[330−332] A sugar core containing methacryl-type macromonomer was also developed.[346]

Lipase is sensitive to a stereochemical factor of the substrate.[362] Since benzyl alcohol is a good initiator, a backbone polymer, poly[styrene-*co*-(4-vinylbenzyl alcohol)] containing 10% hydroxy group ($m/n = 1:9$) was used for grafting of ε-CL (Scheme 58a) and acetylation with vinyl acetate (Scheme 58b). The backbone polymer had M_n values from 8 000 to 13 000. Both reactions were carried out in toluene at 70 °C for 24 h. The ε-CL grafting density was at most 50−60% of the pendant hydroxy group, where M_n

Scheme 58



values of the grafted poly(ε-CL) were around 4000−5000. On the other hand, the acetylation was much easier, reaching 95%. The results suggested that this is not the result of limited accessibility of the backbone but is that of sterical constraints. The unreacted OH group in Scheme 58a can further serve for subsequent reactions. This type of reaction selectivity is impossible by a conventional chemical process. Those results can be extended to selective functionalization of polymers by lipase-catalyzed transesterification, that demands high stereochemical regulation.[363]

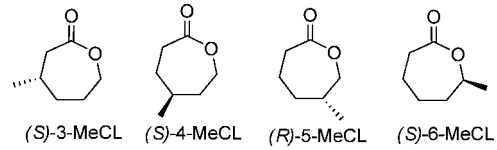
A one-pot, lipase-catalyzed, graft-polymerization of ε-CL and β-butyrolactone (β-BL) onto chitin and chitosan was accomplished in bulk at 70 °C. The ROP of the cyclic monomers was initiated regioselectively from 6-OH group in chitin and from 6-OH and NH groups in chitosan to produce chitin-*graft*-polyester and chitosan-*graft*-polyester, respectively. In the products, both the stem polymer cellulose and the graft polyester are biodegradable.[364]

4.2.1.6. Enantioselective Polymerization. Lipase PS-30 (from lipase PF) induced an enantioselective ROP of α-methyl-β-propiolactone (4-membered) in toluene to produce an optically active (*S*)-enriched (uo to 75% (*S*)) polymer with M_n values from 2000 to 2900 and $[\alpha]^{25}_D$ +12.2° to +19.0° (c = 0.9 g/dL, $CHCl_3$).[289] The enantioselective ROP of 3-methyl-4-oxa-6-hexanolide (MOHEL) was catalyzed by lipase PC in bulk at 60 °C.[261] The apparent initial reaction rate of (*S*)-isomer was seven times larger than that of (*R*)-isomer, indicating that the enantioselective polymerization of MOHEL was induced effectively.

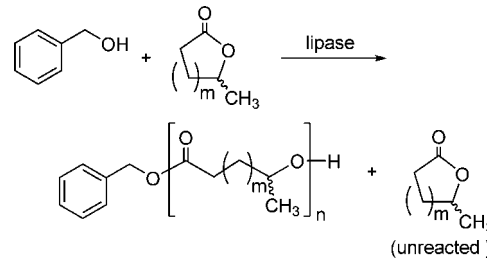
Lipase CA (Novozym 435)-catalyzed enantioselective ROP of substituted ε-CLs (racemic 4-MeCL and 4-EtCL) was achieved in bulk at 45 and 60 °C to give highly (*S*)-enriched poly(4-MeCL) and poly(4-EtCL) with M_n values of 4400 and 5400, respectively, both having enantiomeric purity >95%.[365] Enantioselective ROP of methyl substituted ε-CLs were carried out with Novozym 435 catalyst. A benzyl alcohol-initiated ROP at 45 °C showed (*S*)-selective for 3-MeCL, 4-MeCL, and 6-MeCL and (*R*)-selective for 5-MeCL. 6-MeCL did not propagate in the ROP. Values of the enantiomeric ratio *E* of 3-MeCL, 4-MeCL, and 5-MeCL were 13 ± 4, 93 ± 27, and 27 ± 7, respectively. The enantiomeric excess (ee) value in the polymer of 4-MeCL was 0.88 (Scheme 59).[366] Lipase CA-catalyzed enantioselective ROP of fluorinated lactones in the ring size from 10 to 14 was reported. The corresponding oxyacid gave an optically inactive polyester.[367]

In the lipase CA-catalyzed copolymerization of β-butyrolactone (β-BL) with DDL, *(S)*-β-BL was preferentially

Scheme 59



Scheme 60



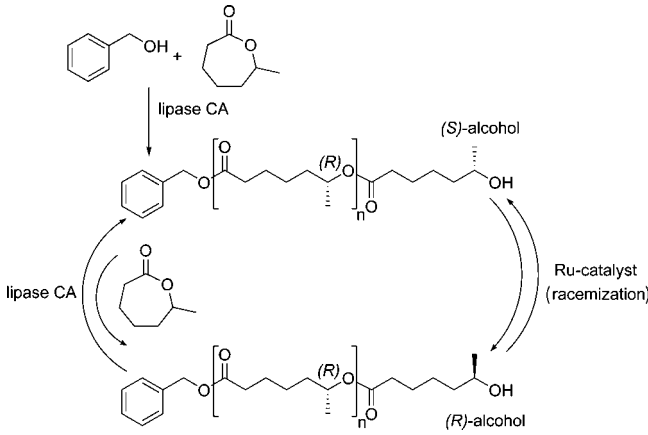
reacted to give the (*S*)-enriched optically active copolymer with ee of β-BL unit = 69% (Scheme 54, p = 1). δ-Caprolactone (6-membered) was also enantioselectively copolymerized with achiral lactones by the lipase catalyst to give the (*R*)-enriched optically active polyesters reaching 76% ee, in which the enantioselectivity was opposite (Scheme 54, p = 3).[352,353]

Lipase CA (Novozym 435)-catalyzed ROP of ω-methylated six lactones was studied for elucidation of the relationships between enantioselectivity and lactone structure (Scheme 60).[368] The rate of polymerization was much affected by the ring size as suggested by k_{cat} values and the enantioselectivity was switched from (*S*)-selective for small (4-, 6-, and 7-membered) lactones to (*R*)-selective for large (8-, 9-, and 13-membered) lactones. From the k_{cat} values (s^{-1}) [for 4-, 6-, 7-, 8-, 9-, and 13-membered (*S*)-enantiomers, 45.7, 7.9, 49.3, 0.01, nd, and nd, respectively, and (*R*)-enantiomers, nd, 7.6, 8.5, 204.4, 10.3, and 23.3, respectively, where nd means not detected], the enantiomeric ratio *E* was very large, in particular, for the large lactones. The selectivity switch was further supported by the molecular modeling studies on the free energy difference between the lactone structure and the active site cavity of the lipase. The lactone takes transoid and cisoid conformations; virtually small lactones for cisoid and large lactones for transoid. ROP of the small cisoid lactones was (*S*)-selective (3-MePL and 6-MeCL) or aselective (5-MeVL). ROP of the larger transoid lactones was (*R*)-selective with very high enantioselectivity. For the intermediate ring sizes, 7-MeHL and 8-MeOL, the significant amount of cisoid conformers present did not affect the enantioselectivity. The interpretation of the enantioselectivity and lactone structure relationship is attractive, but it seems actually not simple, particularly for understanding the intermediate ring sized case.[368] A selectivity switch was also observed in the copolymerization from (*S*)- for 3-MePL (4-membered) to (*R*)-selectivity for 5-MeVL (6-membered) (Scheme 54),[352] 4-alkyl-substituted ε-CL (Scheme 55),[356] and methyl-substituted ε-CL (Scheme 59).[366]

It is to be noted that Novozym 435 did not induce the ROP of LL-lactide but induced the ROP of DD-lactide (DLA) enantioselectively to produce polyDLA of M_n value of 3300 in toluene at a lower reaction temperature of 70 °C for 3 days (Scheme 49).[304]

A new method of iterative tandem catalysis (ITC) was created, by which optically active oligoesters were obtained via ROP of 6-methyl-ε-CL (6-MeCL).[369,370] ITC means a

Scheme 61



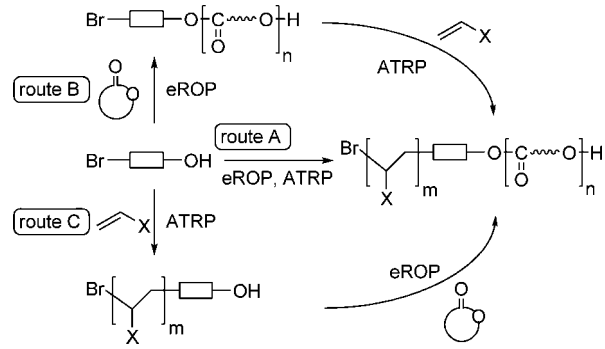
polymerization in which the chain growth is effectuated by a combination of two different catalytic processes that are both compatible and complementary. By combining the lipase CA-catalyzed ROP of racemic 6-MeCL and the Ru-catalyzed racemization of a propagating secondary alcohol in one-pot, optically pure oligoesters were obtained, which was developed based on the dynamic kinetic resolution (DKR) method (Scheme 61). First, lipase CA catalyzes the ring-opening of an (*S*) monomer enantioselectively to give the benzyl alcohol adduct, but the (*S*) alcohol is less favored to react with the monomer via ring-opening and hence the Ru-catalyzed racemization takes place to give a racemic alcohol. Then, an (*R*) alcohol selectively reacts with the monomer to facilitate one monomer-unit elongated. This reaction cycle repeats and ended up with the production of (*R*) oligoesters from the racemic monomer. The cycle could be repeated up to five times, being up to five monomer units of the product oligomers. Since the ring-opening of racemic 6-MeCL by lipase CA catalyst took place only for the (*S*)-isomer, (*R*)-6-MeCL virtually remained unreacted. In principle, this method provides with a flexible tool for obtaining chiral macromolecules from racemic or prochiral monomers.

The simultaneous dynamic kinetic resolution (DKR) of a secondary alcohol was combined with lipase-catalyzed ROP of ε-CL. (*R*,*S*)-1-Phenylethanol (PhE) was used as a model secondary alcohol and incoroporated into poly(ε-CL) under DKR conditions with using lipase CA and a Ru catalyst. A total of 75% of the PhE was incorporated as (*R*)-PhE-poly(ε-CL) with over 99% ee in 23 h at 75 °C in toluene. This methodology could give a simple one-step approach to prepare enantiopure covalently tethered sustained release polymeric formulations of chiral species such as drugs or drug precursors bearing a secondary alcohol group.[371]

4.2.1.7. Chemoenzymatic Polymerization. Enzymes are "green" biocatalysts, in contrast to "chemical" metal catalysts, which are sometimes toxic and not renewable. More importantly, catalysis of these two classes is quite different in function and mechanism but can be mutually compatible to allow the catalysis concurrently in the same reaction system, if the combination of two classes of catalyst is appropriate. With utilizing these advantages of enzymes, chemoenzymatic method has recently been developed for the synthesis of new polymeric materials, in particular, various block copolymers, which are otherwise difficult to prepare.

Multiarm heteroblock star-type copolymers of poly(lactic acid) (PLA) and poly(ε-CL), poly(LA-*co*-ε-CL), were prepared via a chemoenzymatic route. First, ROP of ε-CL was

Scheme 62



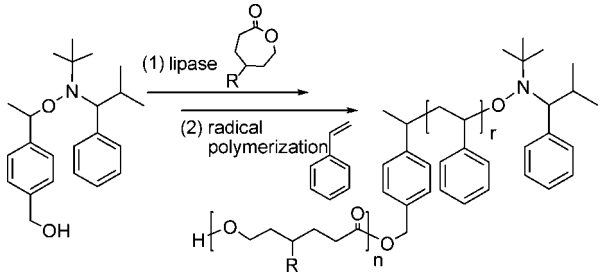
initiated regioselectively from 6-OH of ethyl glucopyranoside by PPL catalyst and the terminal OH was acetylated by using vinyl acetate with lipase catalysis. Second, Sn-catalyzed ROP of lactide was initiated from 2-, 3-, and 4-OH groups to give the copolymer consisting one poly(ε-CL) arm with M_n = 1300 and three PLA arms with M_n of the final product = 11 500.[372]

A new chemoenzymatic method was developed by the combination of an enzymatic polymerization and a chemical polymerization;[373] enzyme (lipase)-catalyzed ring-opening polymerization (eROP) of lactones and atom transfer radical polymerization (ATRP).[374] The combination of two different, consecutively proceeding reactions is referred to as *cascade polymerization*.[366] The method allows a versatile synthesis of block copolymers consisting from a polyester chain and a vinyl polymer chain by using a designed bifunctional initiator, whose reaction routes are generally shown in Scheme 62.[366,373,375−377] For example, in route B an initiator having OH group for eROP and Br atom for ATRP is to be used. Lipase CA (Novozym 435) catalyzed the ROP of ε-CL at 60 °C in toluene to give poly(ε-CL). Then, the poly(ε-CL) having the bromide was isolated and used for the Cu-catalyzed radical polymerization of styrene (St) at 85 °C in 1,4-dioxane to give poly(ε-CL-*block*-St) in a high yield, in which the ε-CL chain exhibited M_n 5.8 × 10^3 and the St chain 15 × 10^3.[373] In one-pot chemoenzymatic cascade synthesis of block copolymers combining lipase catalyzed ROP of ε-CL and ATRP of alkyl methacrylate monomer, reaction factors like effects of a ligand structure of CuBr catalyst on the enzyme catalyst activity were examined in detail. The ATRP system showed an inhibition effect on the enzymatic activity. Methyl methacrylate interfered with eROP by transesterification, whereas *t*-butyl methacrylate was inert.[378]

The principle was extended to an optically active block copolymer synthesis. For example, with using racemic monomer of 4-MeCL (Scheme 59), lipase CA catalyst, and a bifunctional initiator having OH and Br, enantioselective enzymatic ROP of 4-MeCL was induced from the OH group, and then ATRP of methyl methacrylate (MMA) was initiated from the Br atom to afford a block copolymer, poly(4-MeCL-*block*-MMA) having a chiral polyester chain.[366] By optimization of the reaction conditions, side reactions such as a homopolymer formation of poly(ε-CL) could be minimized to <5% with taking an opitimized enzyme drying procedure.[375]

Branched polymers with or without polyMMA chain from a poly(ε-CL) macromonomer were produced by using the chemoenzymatic technique: 2-Hydroxyethyl α-bromoisobutyrate was used as a bifunctional initiator to synthesize a Br

Scheme 63



containing poly(ε-CL) macromonomer, to which a polymerizable end group was introduced by in situ enzymatic acrylation with vinyl acrylate. Subsequent ATRP of the acrylate macromonomer gave branched polymers.[376]

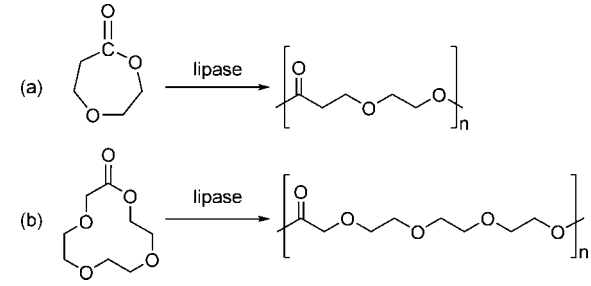
Similarly, the principle was applied for one-pot synthesis of a block copolymer by using a nitroxide mediated radical process. Thus, metal-free poly(ε-CL-*block*-St) was obtained in two consecutive polymerization steps via corresponding route B and in a one-pot cascade approach without intermediate transformation or workup step via corresponding route A. A chiral block copolymer with high enantiomeric excess was obtained by using 4-alkyl ε-CL (4-RCL) and styrene as comonomers, where the first step was a lipase-catalyzed enantioselsctive ROP of 4-RCL at 60 °C and the second was a free radical living polymerization of styrene at 95 °C (Scheme 63).[377]

A spontaneous single-step chemoenzymatic synthesis of block copolymers was facilitated in supercritical carbon dioxide ($scCO_2$) via route A in Scheme 62.[321] For example, the reaction was carried out in $scCO_2$; at 35 °C, 1 500 psi (10.3 MPa) using MMA, ε-CL, Novozym 435, CuCl, and 2,2′-bipyridine. With reaction time of 20 h, a block copolymer, poly(ε-CL-*block*-MMA), of $M_n = 41\ 000$ ($M_w/M_n = 2.11$) was obtained in 60% yields. PolyMMA block part showed $M_n = 10\ 000$ ($M_w/M_n = 1.02$), indicating a living radical polymerization of MMA. Each homopolymer was produced in small amount, less than 10%. These data indicate that the two catalyst systems are robust under the reaction conditions and can tolerate each other. Block copolymer synthesis via routes B and C was also possible.

An amphiphilic block copolymer was prepared from a fluorooctyl methacrylate (FOMA) in $scCO_2$ by a sequential monomer addition technique.[322] A poly(ε-CL) macromonomer initiator was first obtained via eROP induced by the bifunctional initiator given similarly in Scheme 63. A FOMA was then grown from the macroinitiator to produce the product copolymers, poly(ε-CL-*block*-FOMA), consisting poly(ε-CL) chain of M_n values from 3×10^3 to 30×10^3 and polyFOMA chain of M_n values from 5×10^3 to 18×10^3.

For chemoenzymatic synthesis combining eROP and ATRP, 2,2,2-trichloroetahnol was employed as a new initiator. First, CCl_3CH_2O-initiated poly(ε-CL) was prepared by Novozym 435-catalyzed ROP of ε-CL, and then, styrene (St) polymerization was initiated from C−Cl bond cleavage via ATRP to give rise to poly(ε-CL-*block*-St) having the degree of polymerization (DP) of ε-CL/St = 76/56.[379] Instead of St, glycidyl methacrylate (GMA) was used for preparing a new amphiphilic block copolymer, poly(ε-CL-*block*-GMA), via chemoenzymatic technique. Products of two block copolymers possessed M_n of 22 800 with DP values ε-CL/GMA = 68/85 and M_n of 60 700 with DP values ε-CL/GMA = 68/352. They formed polymeric micelles; average hydrodynamic diameter (HD) values were 80−100 nm and 180−250 nm, respectively.[380] With using $-CCl_3$ terminal group, block copolymer of polyDDL and PSt was synthesized by combination of lipase-catalyzed ROP of DDL and ATRP of St monomer, ending up with M_n around 11 000.[381]

Scheme 64



A chemoenzymatic process combining eROP and ATRP was employed for synthesizing heterografted molecular bottle brushes (HMBB) having a complicated structure.[382] In the first step, an approximately 50% homografted polymer, poly((glycidol-*graft*-ε-CL-acetyl)-*co*-glycidol, was obtained via Novozym 435-catalyzed ROP of ε-CL using polyglycidol as a multifunctional initiator. Then, selective acetylation of the hydroxy groups at the graft ends was performed via enzymatic acetylation with vinyl acetate, and the hydroxy groups at the backbone were acylated with 2-bromo-2-methylpropionyl bromide. Finally, MMA or *n*-BuMA grafts were attached by ATRP technique initiated from the bromine atom with CuBr/2,2′-bipyridyl catalyst at 60 °C. The resulting HMBB possessed M_n values (reaction time): 55 000 (75 min) and 83 000 (120 min) for MMA grafts; 60 000 (120 min) and 100 000 (210 min) for *n*-BuMA grafts.

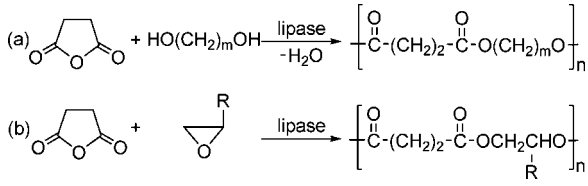
In the chemoenzymatic synthesis of a liquid crystalline polymer, a starting monomer with liquid-crystalline side chains was prepared with Novozym 435 catalyst to produce 6-(4-methoxybiphenyl-4′-oxy)hexyl vinyl hexanedioate.[383] For the synthesis of multifunctional poly(meth)acrylates, functional monomers of (meth)acrylates were derived from Novozym 435-catalyzed transacylation of methyl methacrylate (MMA) and acrylate (MA) with various functional alcohols. These monomers thus prepared were radically polymerized by AIBN initiator to give poly(meth)acrylates via cascade reactions. The obtained polymers possessed hydrophilic, hydrophobic, and cationic nature and can be used for surface coating for various purposes.[384]

4.2.2. Ring-Opening Polymerization of Other Monomers

A poly(ester-*alt*-ether) was synthesized by ROP of 1,5-dioxepan-2-one (DXO) by lipase CA catalyst, giving a high molecular weight polymer up to $M_n = 5.6 \times 10^4$, $M_w = 11.2 \times 10^4$ at 60 °C. The polymerization behaved like a living system (Scheme 64a).[327,385] Poly(1,4-dioxan-2-one) (polyDO) is a desirable biocompatible polymer with good flexibility and tensile strength. Metal-free polyDO aiming medical application was prepared by lipase CA-catalyzed ROP of DO at 60 °C, having $M_w = 41\ 000$.[386] CALB catalyzed the ring-opening copolymerization of DO with PDL in toluene or diphenyl ether at 70 °C for 26 h gave a copolyester of poly(DO-*co*-PDL) with $M_w > 30\ 000$.[299]

Lipase-catalyzed ROP of cyclic diester type lactones, ethylene dodecanedioate (16-membered) and ethylene tridecanedioate (17-membered), was induced at 45−75 °C in bulk to give the ring-opened polyester with M_n up to 4100.[387] A

Scheme 65



cyclic dimer of ε-CL (14-membered) was polymerized by lipase CA catalyst to yield poly(ε-CL) with $M_n = 89\,000$.[268]

A new 12-membered lactone, 2-oxo-12-crown-4-ether (OC), showed a high ROP reactivity by Novozym 435 catalyst to give polyOC ($M_n = 3500$) showing $T_g \approx -40$ °C, soluble in water (Scheme 64b). Copolymerization of OC with PDL tuned the copolymer properties (M_n around 4400−12 200). Although OC polymerized five times faster than PDL, the copolymer showed a random copolymer structure.[388]

Cyclic acid anhydrides underwent the ring-opening addition-condensation polymerization with a glycol by lipase PF catalysis to give a polyester with M_n around 2000 ($M_w/M_n = 1.4$) in good yields. During the reaction the ring-opening, as well as the dehydration, occurred (Scheme 65a).[389] Various cyclic anhydrides, succinic, glutaric, and diglycolic anhydrides, were polymerized by lipase CA catalyst with α,ω-alkylene glycols in toluene at 60 °C to give the polyesters. Under appropriate conditions, M_n reached 1.0×10^4. This ring-opening addition-condensation polymerization involving dehydration proceeded also in water and $scCO_2$.[390]

Biodegradable polyesters were prepared via ring-opening copolymerization of succinic anhydride and various oxiranes (Scheme 65b).[391−393] Other cyclic carboxylic anhydrides include dicarboxylic anhydride, phthalic anhydride, and maleic anhydride because oxiranes glycidyl phenyl ether, benzyl glycidate, and styrene oxide were employed.

4.3. Environmental Aspects

Lipase catalysis is highly selective in all respects, in enantio-, regio-, and chemoselectivities, and can be achieved under mild reaction conditions. Lipase is also active for selective end-functionalization (modification of polymers) with a clean process. The catalyst activity is achieved not only in naturally occurring solvent conditions, mainly in water, but also in pure organic media, in super critical carbon dioxide, and even in ionic liquids, at a wide range of temperature. Among others, it is a nontoxic biocatalyst, which is renewable. It is relatively cheap compared with other enzymes, and recently, a variety of lipase became commercially available.

Because of these characteristics, lipase catalysis has potentials to contribute to environmental problems for maintaining green sustainable society by pursuing *green polymer chemistry*.[8,17,18,20,22,24,25,174,204,363,394,395] Some of the lipase-catalyzed synthesis and end-functionalization of polyesters mentioned above already provided with good examples of green polymer chemistry from the viewpoint of clean-process, energy savings, natural resources, carbon dioxide emission, etc. Despite practical applications of enzyme catalysts including lipase to food industry and chemicals production in various ways, lipase catalyst has scarcely been utilized for the polymer synthesis on a scale for an industrial production.

A possible way of conducting green polymer chemistry is a recycling of polyesters.[161,164] Industrial examples of chemical recycling are few but known like an alcoholysis method of poly(ethylene terephthalate) (PET) and poly(butylene terephthalate). Biobased polymers are expected as an alternative in place of conventional non-biobased polymers. Poly(lactic acid) (PLA) is a good example in this direction. Thus, a new method of chemical recycling of polymers using lipase catalysis was proposed.[268,281,284,396] The principle lies in that the ROP system of lactones by lipase catalysis is reversible between polymers and oligomers, which can be controlled by changing the reaction conditions, i.e., the lactone polymerization gives cyclic oligomers including monomer in a dilute solution and gives higher molecular weight polymers in a concentrated solution which can be degraded to cyclic oligomers again in a dilute solution by the same catalyst. This cycle can be conducted repeatedly in a batch process. The amount of water in the system is important and must be controlled strictly each time.

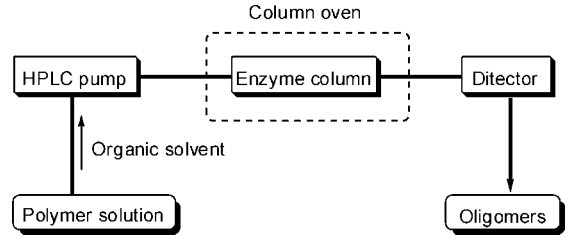


Figure 15. Schematic diagram of continuous degradation system.

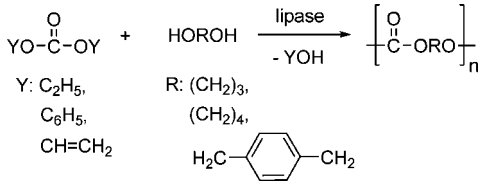
This recycling possibility was extended to diol-diacid type polyesters The molecularly pure cyclic oligomers, monomers, dimers and trimers, were prepared by the lipase-catalyzed degradation of linear three kinds of polyesters, poly(butylene adipate) (PBA), poly(butylene succinate) (PBS), and poly(ε-CL). Then, cyclic oligomers from these polyesters were subjected to ROP by Novozym 435 catalyst in toluene at 60−120 °C for 24 h. The M_w values of the product polyesters, PBA, PBS, and poly(ε-CL), reached 17.3×10^4 from BA monomer, 17.2×10^4 from BS dimer, and 7.9×10^4 from ε-CL dimer, respectively. This cyclic oligomer-linear polyester interconversion by lipase catalysis is a possible way for chemical recycling.[397] In addition, a high molecular weight linear PLA was transformed into cyclic oligomers of 6- to 20-mers in good yields by lipase-catalyzed degradation of PLA in various organic solvents, typically in *o*-xylene at 100 °C.[398]

To reduce the necessary amount of lipase, a continuous degradation system was proposed. By this method biodegradable polyesters were transformed into repolymerizable cyclic oligomers through lipase catalyst packed in a column (Figure 15).[164,399,400] Polyesters such as poly(3-hydroxybutanoate) (PHB), poly(ε-CL), and PBA were examined. For example, when a toluene solution of poly(ε-CL) ($M_n = 13.1 \times 10^4$) was allowed to flow an immobilized lipase CA column at 40 °C, cyclic oligomers ($M_n = 220$, $M_w/M_n = 1.08$) were quantitatively obtained, which could be repolymerized. Instead of toluene, $scCO_2$ will be a better candidate solvent.

5. Synthesis of Polycarbonates, Polyamides, Polyphosphates, and Polythioesters

The title polymers belong to a family of polyesters described in the section 4, and hence, lipases were mainly used as an enzyme catalyst for their synthesis. Analogously, mechanism involving an intermediate (lipase−O−C(=O)−,

Scheme 66



acyl-enzyme intermediate, Scheme 52 and Figure 14) or a corresponding intermediate is considered in all cases for the formation reaction of −O−C(═O)O−, −NH−C(═O)−, −O−P(═O)(OR)O−, or −S−C(═O)− bond.

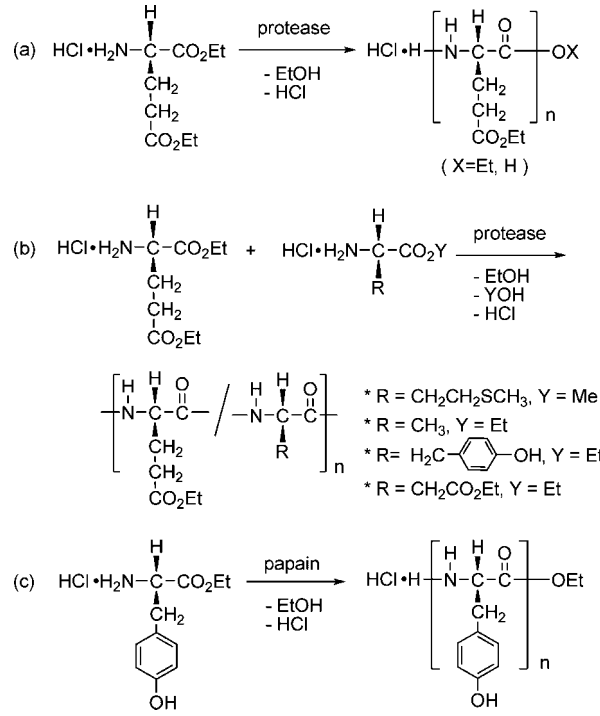
5.1. Polycondensation

Polycarbonates were prepared by lipase catalyzed polycondensation of a carbonic acid diester and a glycol (Scheme 66). The reactions produced aliphatic polycarbonates of molecular weight higher than 4×10^4,[401,402] and aromatic polycarbonates of DP greater than 20.[241] Transesterification between diethyl carbonate and a diol to produce polycarbonates proceeded via two stages; the first to yield oligomers and the second to give higher molecular weight polymers.[403] CALB-catalyzed transesterification among three components, diethyl carbonate, a diester, and a diol, formed aliphatic poly(carbonate-*co*-ester)s with about 1:1 molar ratio of the ester-to-carbonate repeat units. Molecular weight M_w value reached 59 000 at a reaction temperature 90 °C. A carbonate−ester transesterification reaction between poly(butylene carbonate) and poly(butylene succinate) was also catalyzed by CALB at 95 °C to give a block copolymer.[404]

In the synthesis of polyamides [poly(amino acid)s, polypeptides], protease (E.C. 3.4) catalyzed the condensation polymerization and copolymerization of L-glutamic acid diethyl ester hydrochloride (GADE), for which papain (E.C. 3.4.22.2), bromelain (E.C. 3.4.22.4), and α-chymotrypsin (E.C. 3.4.21.1) showed high catalytic activity. Proteases catalyze not only hydrolysis of peptide bonds but also peptide bond formation under appropriate conditions to give polypeptides, whereas the product characterization was not fully performed.[8] A papain-catalyzed polymerization of GADE in phosphate (pH 7) at 40 °C for 3 h, giving rise to a well-characterized poly(α-peptide) with $M_n = 1200$ in 61% yields; specific rotation $[\alpha]_{589}$ was −5.1° (DMSO, $c = 10$) (Scheme 67a).[405] No γ-peptide unit was formed, indicating the perfect regioselectivity of α-structure. In relation to these results, the papain-catalyzed polymerization of γ-methyl-L-glutamate did not occur. Copolymerization of GADE monomer with several L-amino acid esters including methionine methyl ester gave for the first time copoly(amino acid)s in good yields under similar reaction conditions (Scheme 67b). In a similar protease-catalyzed polycondensation of L-glutamic acid diethyl ester hydrochloride, effects of reaction conditions like pH and ionic strength on the polymer yield, molecular weight, and catalyst stability were examined.[406]

Alkanophilic protease-catalyzed polycondensation of diethyl L-aspartate in bulk produced a polypeptide having a mixed structure of α- and β-peptide linkages.[407] Diethyl L-aspartate was polymerized by using a bacterial protease from *Bacillus subtilis* as catalyst in organic solvent like acetonitrile at 40 °C for 2 days, affording poly(ethyl-L-aspartate) with M_w up to 3700 in 85% yields. The polymer possessed an exclusive α-linked structure and showed a good biodegradability.[408]

Scheme 67



Papain-catalyzed polycondensation of L-tyrosine ethyl ester hydrochloride was conducted in phosphate buffer (pH 7) at 40 °C for 3 h, to give a polymer of α-peptide structure, poly(tyrosine), with molecular weight about 2000 (Scheme 67c). Poly(tyrosine) was soluble in DMF, DMSO, and alkaline solution but was insoluble in acetone, chloroform, toluene, THF, and water. The product poly(tyrosine) after 72 h showed the formation of the globular particle in the diameter larger than 50 μm (Figure 16A). The cross section

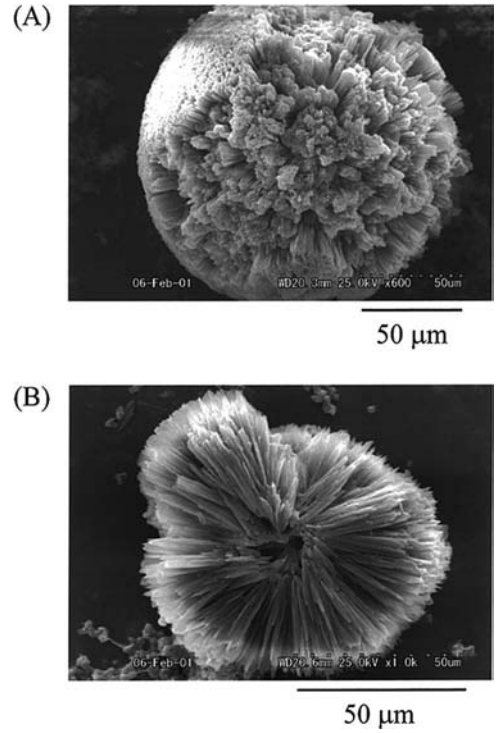
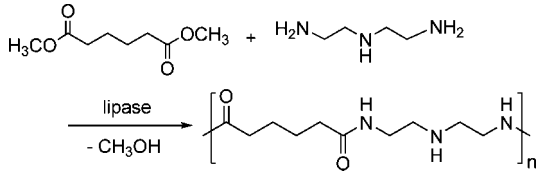
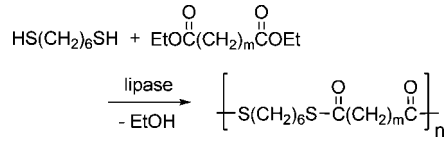


Figure 16. Scanning electron microscopy (SEM) images of (A) poly(tyrosine) obtained after 72 h, and (B) the cross section of the fragment. (Reprinted with permission from ref 409. Copyright 2002 American Chemical Society.)

Scheme 68



Scheme 69



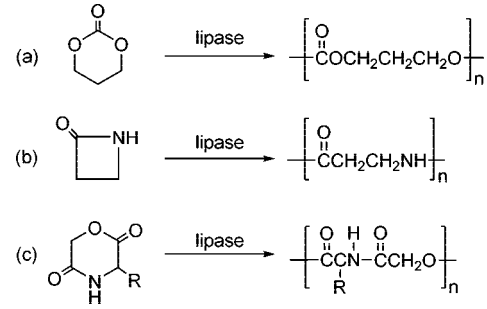
of the fragment showed that rodlike crystals radially originated from the center (Figure 16B).[409] This morphology of poly(tyrosine) is characteristic of the enzymatic polymerization of the monomer, since such a globular crystal was not observed before in the recrystallization of poly(α-amino acid)s including poly(tyrosine).[410]

Oligopeptides having various functions have been synthesized extensively using proteases as catalyst via condensation reactions in either a thermodynamically controlled or a kinetically controlled manner.[411] For examples, aspartame (Asp-Phe) by thermolysin,[412] oxytocin (Cys-Tyr-Tyr-Ile-Pro-Leu-Leu-Gly) by papain, thermolysin, and chymotrypsin,[413] and somatostatin (cyclic, -Ala-Gly-Cys-Lys-Phe-Phe-Trp-Lys-Thr-Phe-Thr-Ser-Cys-) by thermolysin, and chymotrypsin,[414] were prepared as typical biologically active peptides.

Various polyamides were prepared by an enzyme-catalyzed polycondensation.[415] For the synthesis of polyaminoamides from dicarboxylic acids and amines, several proteases like chymotrypsin, trypsin, subtilsin, and papain were screened, but only lower molecular weight polymers were obtained. It is to be noted that lipase catalyzed the polycondensation. Among the lipases examined, Novozym 435 and *Mucor miehei* showed the highest catalytic activities. Typically, the reaction of dimethyl adipate and diethylene triamine produced the polyaminoamide at 60−100 °C with M_w = 8000−9000 (Scheme 68). A chemical method to prepare similar polymers needs a higher reaction temperature like 180 °C. Other carboxylic acids such as malonic acid and fumaric acid were used. As amines, triethylene tetramine, tetraethylene pentamine, and triethylene glycol diamine were also employed. The enzymatic method gave polymers with less branching structure compared with a chemical method.

An aliphatic poly(thioester) was synthesized by direct polycondensation of 11-mercaptoundecanoic acid (11-MU) with lipase CA catalyst in bulk in the presence of molecular sieves. The reaction at 110 °C for 48 h produced poly(11-MU) with M_w of 34 000 in high yields. The T_m value of 104.5 °C was about 20 °C higher than that of the corresponding polyoxyester. Poly(11-MU) was readily transformed by lipase into the cyclic oligomers mainly of the dimer, which were readily repolymerized by lipase via ROP as a sustainable chemical recycling.[416] A polythioester was prepared by lipase CA catalyst via the direct transesterification of 1,6-hexanedithiol and a diacid diester with eliminating ethanol in bulk in the presence of molecular sieves (Scheme 69, m = 1−8). The product polythioester possessed $M_w \approx 1.0 \times 10^4$. Both the melting point and crystallization temperature were higher than those of the corresponding polyoxyacids.[417]

Scheme 70



A copolymer of an ester-thioester structure was enzymatically synthesized. The lipase CA-catalyzed copolymerization of ε-caprolactone (ε-CL) with 11-mercaptoundecanoic acid or 3-mercaptopropionic acid under reduced pressure produced the copolymer with molecular weight higher than 2 × 10^4. The transesterification between poly(ε-CL) and 11-mercaptoundecanoic acid or 3-mercaptopropionic acid also took place.[418]

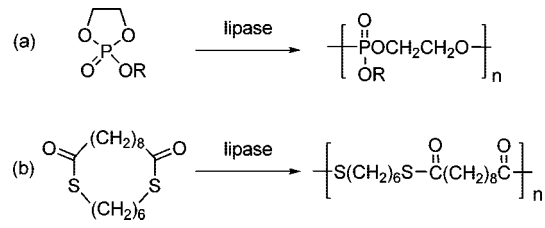
5.2. Ring-Opening Polymerization

In 1997, a polycarbonate synthesis from 1,3-dioxan-2-one (trimethylene carbonate, TMC) was first reported (Scheme 70a);[419,420] lipase CA efficiently catalyzed the ring-opening polymerization (ROP) of TMC under mild reaction conditions at 70 °C to give the product having M_n higher than 10 000. At a higher temperature of 100 °C, the ROP with PPL catalyst afforded a polycarbonate of higher $M_w \approx 1.6 \times 10^5$.[421] It is to be noted that no carbon dioxide was liberated during the polymerization, in contrast to a chemical anionic polymerization which often involves the liberation of carbon dioxide to produce a non-carbonate unit.

An enantiomerically pure functional polycarbonate was prepared from a new 7-membered cyclic carbonate monomer having a ketal group, that was derived from naturally occurring L-tartaric acid. Lipase-catalyzed ROP of the monomer was induced at 80 °C in bulk, to afford the polycarbonate with $M_n = 1.55 \times 10^4$ (M_w/M_n = 1.7). Optically active polycarbonate was effectively obtained by Novozym 435 catalyst. Deprotection of the ketal group resulted in hydroxy group functionality in the carbonate chain. The polycarbonate is considered to have potentials for various biomedical applications.[422] ROP of cyclic carbonate oligomers and their ring-opening copolymerization with lactones were catalyzed by lipase CA, producing corresponding polycarbonates.[423] Ring-opening copolymerization of a substituted TMC with 1,4-dioxan-2-one or with TMC by lipase catalyst was also reported to give a poly(carbonate-*co*-ester) or a substituted poly(carbonate).[424,425]

A degradable polycarbonate copolymer for pH-dependent controlled drug release with micelle formation was synthesized via chemoenzymatic route.[426] Taking into account the toxicity of the catalyst, degradability with pH-change, amphiphilicity of the chain, and toxicity of the degraded products, a triblock copolymer of ABA type was designed, where A block is poly(trimethylene carbonate) (PTMC) (Scheme 70a) and B block is poly(PEG-*co*-cyclic acetal) (PECA). PECA is an α,ω-glycol synthesized chemically, and lipase CA-catalyzed polymerization of TMC was induced from the glycol OH groups to give the triblock copolymer, which was shown to form micelles and to be a drug carrier biomaterial.

Scheme 71



2-Azetidinone (β-lactam) was polymerized via Novozym 435-catalyzed ROP to afford poly(β-alanine) with the degree of polymerization 8, having a linear structure (Scheme 70b). The reaction produced cyclic side products.[427] Similarly, ROP of substituted β-lactams was performed with lipase catalyst.[428]

A poly(ester-*alt*-amide) copolymer was prepared by the lipase-catalyzed ROP of a six-membered cyclic depsipeptide, 3-(*S*)-isopropylmorpholin-2,5-dione (IPMD), in bulk at 100 and 130 °C, ending up with the polymer having M_n up to 30 $\times$ 10^3 (Scheme 70c). Lipase PS and PPL showed a high catalytic activity, but Novozym 435 was almost inactive for ROP of the monomer. PPL (Porcine pancreatic lipase) catalyzed a ring-opening copolymerization of IPMD with DL-lactide at 100 °C to afford a copolymer with M_n around 1 $\times$ 10^4 and also ROP of these monomers gave the respective homopolymers.[429−431] A monomer of MD derivative 6-*(S)*-methylmorpholin-2,5-dione was also polymerized by lipase catalyst.[432] ROP of a large cyclic ester-urethane oligomer was achieved by lipase CA to yield a poly(ester-urethane) with M_w up to 101 000.[282]

Polyphosphates are useful materials for various applications such as flame retardation, plasticizing, and biomedical utilizations. Lipase-catalyzed ROP of a cyclic phosphate was achieved in bulk with PPL (Scheme 71a). The product polyphosphate had M_n value up to 5800.[433,434]

A new class of biopolyesters having a thioester linkage S−C(═O)− were prepared; lipase CA-catalyzed ring-opening addition-condensation polymerization between ε-CL and a mercaptoalkanoic acid under vacuum produced poly(ε-CL) containing thioester groups in the backbone. The product polyester reached to M_w 22 000.[416,418] A cyclic monomer of 1,6-hexanedithiol-sebacate (18-membered ring) was prepared and polymerized via ROP catalyzed by lipase CA in bulk in the presence of molecular sieves at various temperatures to give a poly(thioester) (Scheme 71b). The highest M_w of 1.2 $\times$ 10^5 was obtained at 120 °C for 48 h. A corresponding ester monomer (18-membered) was copolymerized with the thioester monomer, giving rise to poly(thioester-*co*-ester). Physical properties of these polymers were examined and characterized.[435]

6. Synthesis of Polyaromatics

Polyaromatics are widely found in natural biomacromolecules (biopolymers) such as lignin, oriental lacquer, and flavonoid compounds (see Figure 1). These materials serve as a structural component giving physical strength to living systems and a physiologically active component in living cells.

There are several classes of oxidoreductase enzymes, which are involved in metabolism of living system. Typically, peroxidase (EC 1.11.1.7) contains Fe at the active site (Figure 17a), whereas laccase (EC 1.10.3.2: Figure 17b), tyrosinase (polyphenol oxidase, EC 1.10.3.1), and bilirubin oxidase (EC 1.3.3.5) contain Cu at the active site(s). These enzymes have been reported to serve as catalyst for in vitro oxidative polymerization of phenolic and aniline compounds, where hydrogen peroxide or oxygen gas (air) is often used as an oxidant. The reaction mechanism of the peroxidase seems well established to involve three oxidation states of Fe, following its reaction with hydrogen peroxide and the subsequent reaction with an aromatic substrate.[436] The reaction proceeds via oxidative coupling between radical species.[437−440]

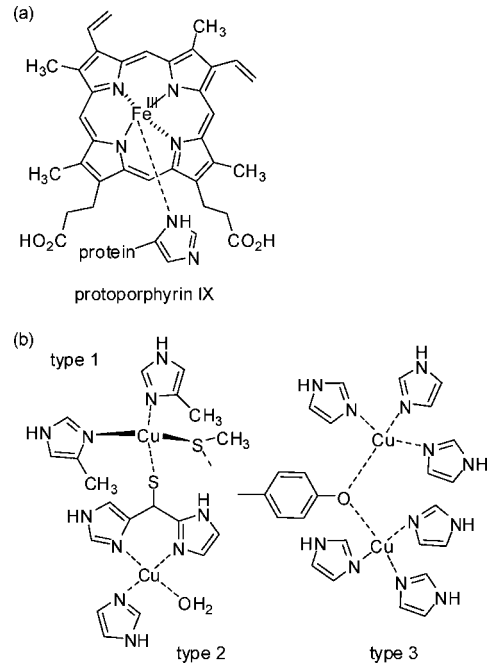


Figure 17. Structures of active site vicinity of (a) horseradish peroxidase (HRP) and (b) laccase.

6.1. Oxidative Polymerization of Phenolic Compounds

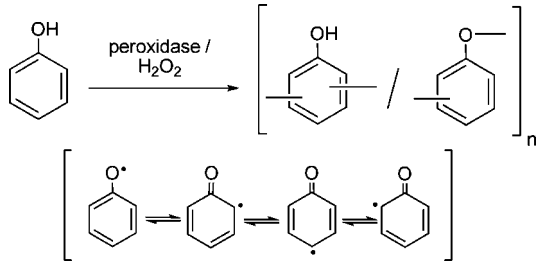
Various phenolic compounds exist in nature; they play an important role in involving metabolism for maintaining the living life. Tyrosine, one of natural amino acids, is a phenol derivative. Flavonoids, such as catechin and rutin, are called "polyphenols", which contain a catechol structure. Therefore, polyphenols are to be distinguished from the polymerized phenols. Here, it is categorized in two classes; phenols are compounds having one phenolic OH group, and polyphenols are those having more than two phenolic OH groups in the aromatic ring.

Polyphenols like catechin attracted much attention through papers, reporting that polyphenols contained in red wine or green tea are effective as an anticancer agent, which was considered because of their antioxidant action.[441,442] They exhibit also anticarcinogenic, antimicrobial, and anti-inflammatory properties, which involve potential benefits in preventing many diseases.

6.1.1. Polymerization of Phenols

6.1.1.1. Unsubstituted Phenol. Currently, polymeric matarials widely produced commercially from phenolic compounds are a phenol/formaldehyde resin (Bakelite) for over the century and poly(2,6-dimethyl-1,4-phenylene oxide) (PPO) for half a century. They possess many desirable properties as an engineering plastic; however, the former

Scheme 72



resin has a defect in the use toxic formaldehyde. Therefore, other phenolic polymers are expected to appear for new materials.[443]

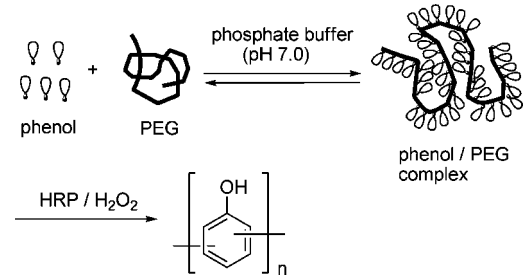
Oxidation polymerization of unsubstituted phenol was examined by using Fe- or Cu-compounds as oxidizing agent; however, the product polymers were insoluble and the structure was uncontrollable.[444] Peroxidase-catalyzed polymerization of phenol, on the other hand, was induced to proceed under mild reaction conditions, giving rise to soluble polyphenol having C−C and C−O coupling units with M_n of 3000−6000 (Scheme 72).[439,445−449] The catalysts include horseradish peroxidase (HRP) and soybean peroxidase (SBP), the reaction of which involves the one-electron oxidation of phenol in the presence of H_2O_2 via oxy- or carbon-radical intermediate shown in resonance forms in Scheme 72, with co-producing H_2O. It was important to select the reaction solvent using an aqueous buffer alcohol (methanol or ethanol). The content of oxyphenylene unit (32−59%) was controlled by varying the methanol amount. The polymer solubility increased with increasing the oxyphenylene unit content.[448] The copolymerization of phenol with a small amount of 2,4-dimethylphenol controlled the molecular weight, because the latter monomer has the only two reactive positions at oxygen and 6-carbon.[450]

HRP-catalyzed polymerization of phenol was performed in an aqueous buffer solution with addition of a catalytic amount of 2,6-di-*O*-methyl-*β*-cyclodextrin, giving rise to soluble polyphenol with M_n around 1000−1400 in high yields. The small amount of the cyclodexitrin was incorporated into the product polymer.[451] In a dispersion system of 1,4-dioxane/buffer mixture, the HRP-catalyzed polymerization of phenol formed relatively monodisperse polymer particles, typically a diameter around 250 nm, where poly(ethylene glycol), poly(vinyl alcohol) or poly(methyl vinyl ether) was used as stabilizer. Substituted phenols like *m*-cresol, *p*-cresol, and *p*-phenylphenol also gave such particles of submicrometer size.[452,453] Thermal treatment of these particles at 1000 °C under nitrogen afforded uniform carbon particles.

With using a template of poly(ethylene glycol) monododecyl ether for the HRP-catalyzed polymerization of phenol in water, the regioselectivity for the phenylene unit was much increased in close to 90%. The regioselectivity enhancement is speculated to be caused by an oriented alignment via hydrogen bonding interaction of phenolic OH groups and ether oxygen atoms of the template (Scheme 73). The polymer was obtained in high yields as a precipitate complexed with the template molecules.[454,455]

In addition, the HRP-induced polymerization of phenol was examined in water with employing amphiphilic triblock copolymers of poly(ethylene glycol) (PEG)-poly(propylene glycol) (PPG)-poly(ethylene glycol) (PEG) (Pluronic) as template. The polymerization using Pluronic F68 (EG_{76}-PG_{29}-EG_{76}) template gave a homogeneous reaction mixture and produced ultrahigh molecular weight polyphenol; $M_w > 10^6$, which was complexed with the template molecules.[456] The absolute molecular weight measurement with a light scattering detector showed M_w value reaching 3.2×10^6. The regioselectivity of the phenylene unit reached 87%. The reason of urtrahigh molecular weight and regioselectivity of the product is considered similarly to effective formation of phenol/Pluronic complexes, which further polymerized between the complexes in this case. The synthesis did not use organic solvents, hence being environmentally benign system. The products are claimed to be useful for potential applications of high-performance phenolic polymers.

Scheme 73



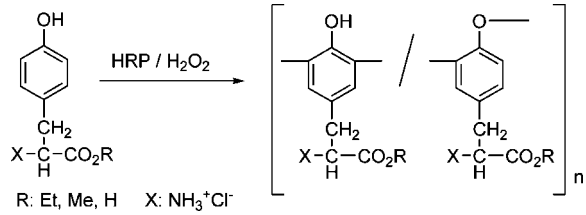
Carbon nanotubes (CNTs) were recently used as template for the HRP-catalyzed polymerization of phenol in water for functionalization of the CNT surface. The polymerization was conducted in the presence of the *p*-hydroquinone (HQ)-linked CNTs, and hence, polyphenol was grafted through the HQ moiety on the CNT surface via the polymerization. The phenol monomer was regioselectively polymerized to possess mainly the thermally stable oxyphenylene units. The high regioselectivity was considered to be the result of the shielding of ortho or para position of phenol from attack by adsorption onto the surface of CNTs.[457]

Phenol was polymerized in a novel bienzymatic system (glucose oxidase + HRP). The system induced the polymerization in the presence of glucose without the addition of hydrogen peroxide to produce the polymer. Hydrogen peroxide was formed in situ by the oxidation of glucose catalyzed by glucose oxidase, which acted as oxidizing agent.[458]

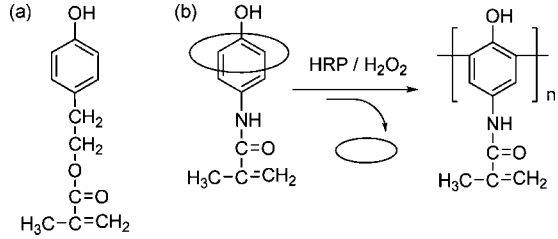
6.1.1.2. Substituted Phenols. *para*-Alkylphenols were polymerized in an aqueous 1,4-dioxane solution by HRP catalysis to give the polymers with M_n of several thousands, whereby the polymer yield increased as *n*-alkyl chain length increased from 1 to 5.[459,460] All alkylphenol isomers were oxidatively polymerized by HRP catalyst, where only *p*-isopropylphenol was polymerized among o-, m-, and p-isomers.[461] Products poly(*p*-alkylphenol)s often involve solubility problem; however, a soluble *p*-ethylphenol with molecular weight less than 1000 was obtained in aqueous DMF.[462]

The oxidative polymerization of substituted phenols much depends on the structure of the phenols with respect to reaction behaviors, product properties, solvent compositions, and enzyme origin. In the polymerization of p-substituted phenols the reactive para-position is blocked and hence the product structure becomes simpler. The product structure from *p*-methylphenol (*p*-cresol) and *p*-propylphenol was studied in details with NMR technique. A coupling mechanism of *p*-cresol was discussed from the structure of dimers produced at the initial stage of polymerization.[463,464] *p*-*t*-Butylphenol and other various p-substituted phenols were

Scheme 74



Scheme 75



polymerized in a mixture of organic solvent and buffer, and structures and biodegradable properties of products were examined.[465−467] Polymer particles of submicrometer size from *m*-cresol, *p*-cresol, and *p*-phenylphenol were obtained in a dipersion system.[452,453]

Oxidative polymerization of a natural phenol derivative, hydroquinone-β-D-glucopyranoside (arbutin), was achieved using peroxidase catalyst in a buffer solution, yielding water-soluble polymers with molecular weight ranging from 1600 to 3200. Acidic deglycosylation of the resulting polymer afforded a soluble poly(hydroquinone), which may be of structure of C−C coupling at the ortho-position.[468] Chemoenzymatic synthesis of poly(hydroquinone) from 4-hydroxyphenyl benzoate was reported, whose structure was different from that obtained from arbutin.[469]

A natural phenolic monomer is tyrosine ethyl ester or methyl ester, which was polymerized using HRP catalyst in an aqueous buffer solution. Poly(tyrosine ester) was obtained from both entantiomeric forms and racemic mixture, with molecular weight between 1500 and 4000. The polymer structure was of mixture of phenylene and oxyphenylene units (Scheme 74). The poly(tyrosine ester) was further converted into a new class of poly(tyrosine) by alkaline hydrolysis of the ester groups. The resulting poly(amino acid) is different from the peptide-type poly(tyrosine) and soluble only in water. In addition, the oxidative homopolymerization of *N*-acetyltyrosine and the copolymerization of *N*-acetyltyrosine with 4-hydroxyphenyl-β-D-glucopyranoside (arbutin) were catalyzed by HRP.[409]

HRP-catalyzed reactions proceed involving radical species, yet took place chemoselectively. HRP-catalyzed oxidation polymerization of a phenol derivative having methacryloyl group (Scheme 75a) occurred only at the phenol moiety, and not at the vinyl group. The product polymer with $M_n \approx 1700$ having methacryloyl group was soluble in an organic solvent and cured thermally, as well as photochemically. In contrast, the monomer without phenolic OH behaved as a vinyl monomer with HRP catalyst.[470]

Phenol moiety-containing vinyl monomers like 4′-hydroxy-*N*-methacryloyl anilide, *N*-methacryloyl-11-aminoundecanoyl-4-hydroxy anilide, and 4-hydroxyphenyl-*N*-maleimide were chemoselectively polymerized at the phenol moiety with HRP catalyst (Scheme 75b). All monomers were used for the polymerization in the form of their cyclodextrin complexes in an aqueous buffer. The resulting phenolic polymers having the vinyl group acted as macromonomers, which were copolymerized subsequently with methyl methacrylate or styrene.[471,472] The polymers were subsequently cross-linked by a radical initiator.[473]

Catalysts HRP and SBP oxidatively polymerized bisphenol A to give a soluble polymer in an aqueous organic solvent.[474] The polymer was composed of a mixture of phenylene unit and oxyphenylene unit, having $M_n = 1000-4000$ ($M_w > 10^4$). It was thermally cured at 150−200 °C to improve the thermal stability. The reaction with an epoxy resin produced an insoluble polymer with high thermal stability, suggesting a possible replacement for the phenol resin without using formaldehyde.

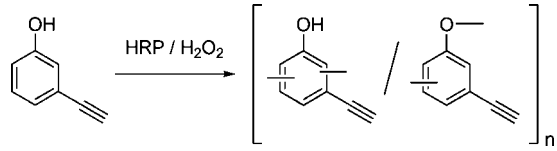
Instead of HRP, *Coprinus cinereus* peroxidase (CiP), a fungal peroxidase, was used for the polymerization of bisphenol A in aqueous 2-propanol solution.[475] With varying the solvent composition from 20 to 50% of 2-propanol in the polymerization at 20 °C for 5 h, the polymer yields and M_n (polydispersity $= M_w/M_n$) values were from 88% to 59% and from 1 130 (1.4) to 18 600 (3.2), respectively. The phenylene unit and oxyphenylene unit ratio of the products was between 65/35 and 58/42. The polymer was mixed with a diazonaphthoquinone derivative to form a film and applied to a photoresist on the silicone wafer by UV irradiation. A sample having a molecular weight around 3 000 gave sharply contrasted patterns.

HRP-catalyzed polymerization of 4,4′-dihydroxydiphenyl ether in an aqueous methanol afforded α,ω-hydroxy-oligo(1,4-oxyphenylene)s in moderate yields.[476] During the reaction, hydroquinone was formed. An unusual mechanism involving the redistribution or rearrangement of the quinone−ketal intermediate with liberating hydroquinone was postulated.

Oxidative polymerization of phenols was conducted not only in the monophasic solvents but in interfacial solvents such as micelles and reverse micelles to give *p*-ethylphenol particles, and biphasic and Langmuir trough systems.[477] 4-Phenylphenol was polymerized in an aqueous surfactant solution, yielding a polymer with relatively narrow molecular weight.[478] HRP-catalyzed reaction of water insoluble phenolic compounds was examined in an ionic liquid of 1-butyl-3-methylimidazolium tetrafluoroborate/water mixture. The catalytic activity in up to 90% ionic liquid in water retained only at pH values higher than 9.0. A high regioselectivity of reaction was noticed; 4-phenylphenol exclusively produced a C−C coupling product, 2,2′-bi-(4-phenylphenol).[479]

HRP-catalyzed oxidative polymerization of 4-hexyloxyphenol was conducted in isooctane solvent, a first example of a total organic solvent system. In order to achieve a homogeneous reaction system in isooctane, HRP was modified by ion-pairing with an anionic surfactant aerosol AT (AOT) and *t*-butyl hydroperoxide was used as oxidant instead of hydrogen peroxide. The product polymer was considered to have a structure via ortho−ortho C−C coupling, with a molecular weight around one thousand. The reaction showed a turnover number about 5 000 μmol substrate converted/μmol HRP.[480] A first diazosulfonate polymer was prepared via HRP-catalyzed polymerization of 4-hydroxybenzenediazosulfonate monomer, where a water-soluble polymer with $M_n = 3000$ was produced.[481] An urushiol analogue phenolic monomer was prepared and oxidatively polymerized by HRP/H_2O_2 or Fe-salen catalyst to produce a prepolymer, and

Scheme 76



further curing thermally or with Co-catalyst yielded cross-linked film (*artificial urushi*) with high gloss surface (vide infra).[482]

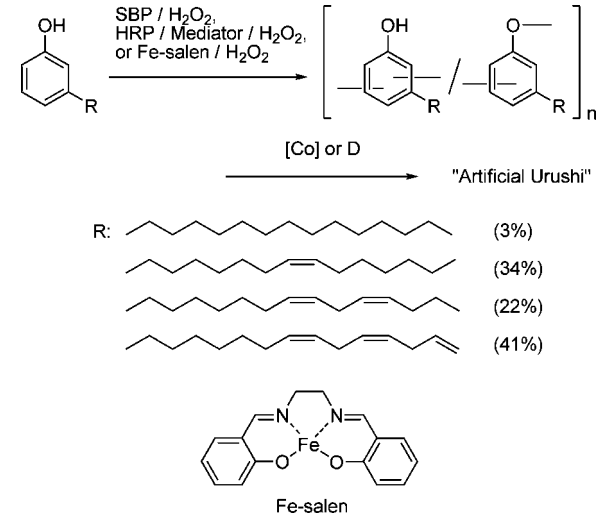
A phenolic polymer from 4-(2-aminoethyl)phenol (tyramine) was prepared in capsules.[483] HRP loaded capsules were first formed via layer-by-layer assembly of polyelectrolytes [poly(sodium 4-styrenesulfonate)/poly(allylamine hydrochloride)]. The selective permeability of the capsule walls allowed the monomer to penetrate and the enzymatic oxidation polymerization of tyramine took place, while the product polymers and HRP left in the capsule interior. Capsule diameter may be controlled in the range from 100 nm to tens of micrometers. Since the monomer tyramine is nonfluorescent and the polymerized tyramine became fluorescent, this biocatalytic method provided a way to synthesize functional materials in the microcapsules and to modify permeation properties of the microcapsule walls. The principle can be applied widely for various enzymes and monomers for functional capsules. Also, HRP catalyzed the polymerization of 4-hydroxyphenylacetic acid (HPA) on the polyelectrolyte capsule wall, on which HRP was assembled with a layer-by-layer technique as both a catalyst and a coupling template.[484] The phenolic polymer formed layers of 70−200 nm thickness and modified the permeability properties of the capsule walls.

meta-Substituted phenols were polymerized with HRP or SBP catalyst in aqueous methanol, giving rise to soluble polymers with a glass transition temperature (T_g) higher than 200 °C. HRP effectively catalyzed the phenol polymerization having a small *m*-substituent monomer, whereas SBP allowed the polymerization of a larger substituent monomer like *m*-isoproryl- or *m*-*t*-butylphenol in a higher yield, which is probably because of a larger geometrical size of the SBP active site. These enzymatically synthesized polyphenols were applied to positive-type photoresists for printed wire boards, because of their high solubility toward alkaline solution and high thermal stability.[485,486] Various m-substituted phenols were oxidatively polymerized in an aqueous buffer in the presence of an equimolar amount of cyclodextrins to give product polymers in high yields.[487] Self-association behaviors of *m*-cresol in a mixed solvent of aqueous buffer and organic solvent were examined, in particular, on the influence of water content, in relation to the HRP-catalyzed polymerization behaviors.[488]

A phenol having an acetylenic group at meta-position was chemoselectively polymerized by HRP catalyst to give a phenolic polymer, despite the possibility of the oxidative reaction of the acetylenic group (Scheme 76).[489] The resulting polymer was very reactive and cured at a lower temperature. It was further converted to carbonized polymer in a much higher yield than enzymatically synthesized poly(*m*-cresol) and is expected as a reactive starting polymer for potential applications.

Cardanol is a main component derived from cashew nut shell liquid (CNSL) and a phenol derivative having mainly the meta-substituent of a C15 unsaturated hydrocarbon chain with one to three double bonds as the major. It has various

Scheme 77

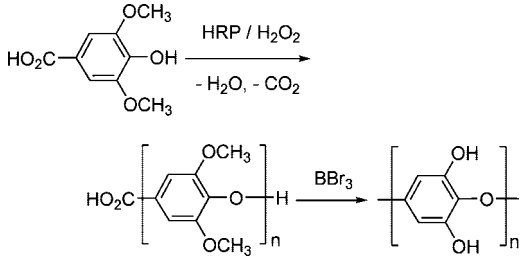


potentials for industrial utilizations such as resins, friction lining materials, and surface coatings. Therefore, development of new applications of cardanol is attractive for "green polymer chemistry".[8,17,18] A new cross-linkable polymer was synthesized by the SBP or Fe-salen catalyzed polymerization of cardanol (Scheme 77), where Fe-salen (Fe(II)-*N*,*N*′-ethylenebis(salicylideneamine)) was regarded as a model complex of the peroxidase.[490−494] The product polymer with molecular weight 2000−4000 was soluble in a polar organic solvent and possessed the unsaturated group in the side chain. Poly(cardanol) was further cross-linked by thermal treatment or by Co-naphthenate catalyzed oxidation, which involves a radical reaction of double bonds. The cross-linked poly(cardanol) showed a tough and hard property as a film with high gloss surface and is regarded as "artificial urushi" because of a close structural resemblance with natural urushi, a traditional Japanese lacquer.[495] In place of phenol ring of cardanol, a naphthol ring was introduced; then the cross-linked film became harder[482,496] With the addition of a redox mediator, such as phenothiazine-10-propionic acid, HRP became active to oxidatively polymerize cardanol, giving the polymer similarly to the above SBP catalyst.[497] In addition to SBP, a fungal peroxidase from *Coprinus cinereus* (CiP) was found effective for the cardanol polymerization, whose catalyst activity in a mixture of buffer/alcohol was close to that of SBP to produce a cross-linkable poly(cardanol) with $M_n = 3000-4100$.[498]

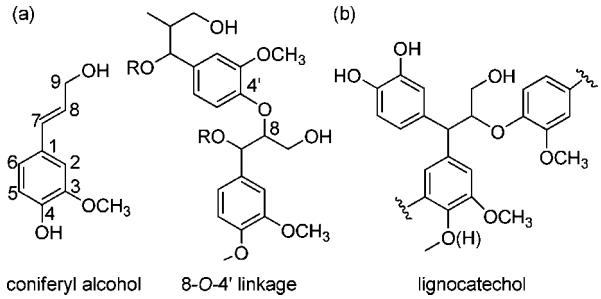
ortho-Methoxyphenols (apocynin, vanillin, and 4-methylguaiacol) were polymerized by SBP catalyst in aqueous buffer using 30% H_2O_2 as oxidant. The products were a variety of oligophenols (dimers to pentamers) and some of their oxidation products, including quinones and demethylated quinones. Some of these are considered to serve as biologically important compounds with therapeutic potencials.[499] An enzyme-activity assay reaction of HRP catalysis using the guaiacol oxidation confirmed new oxidation products of guaiacol trimers in addition to the hitherto known dimeric products, which suggested the accuracy of the current HRP-activity assay method to be reconsidered.[500]

HRP-catalyzed polymerization of 2,6-dimethylphenol afforded poly(phenylene oxide) (PPO) consisting exclusively of 2,6-dimethyl-1,4-oxyphenylene units,[501] which is similar in structure to a widely used high-performance engineering plastic PPO having T_g value of ∼210 °C.[444]

Scheme 78



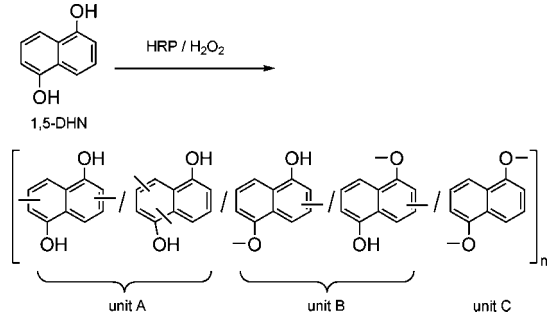
Scheme 79



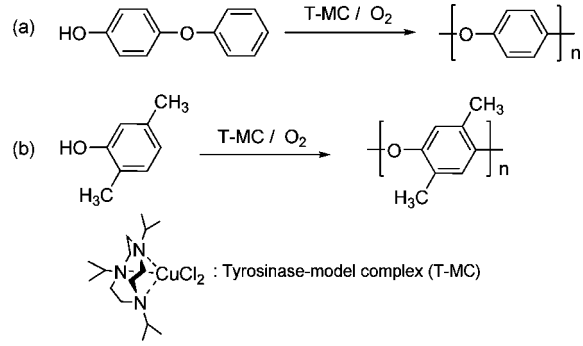
An *o*-disubstituted phenol, syringic acid (3,5-dimethoxy-4-hydroxybenzoic acid) derived from plants was polymerized by HRP or SBP catalyst to produce poly(2,6-dimethoxy-1,4-oxyphenylene) having carboxyl group at the one end and hydroxy group at the other, with liberating carbon dioxide and hydrogen as water during the polymerization. The molecular weight reached 1.5×10^4 with SBP catalyst. The mechanism of the polymerization was well discussed.[502] Demethylation of the product polymer by boron tribromide catalyst gave a new polymer, poly(2,6-dihydroxy-1,4-oxyphenylene), which is thermally stable below 300 °C under nitrogen.[503] Two different mass spectrometric techniques (MALDI-TOF MS and ESI-FTICR MS) were used for the structure study of the products of laccase-catalyzed oxidation polymerization of 2,6-dimethylphenol and syringic acid (3,5-dimethoxy-4-hydroxybenzoic acid).[504]

Coniferyl alcohol (4-hydroxy-3-methoxycinnamyl alcohol, ConA) is a phenolic lignin monomer (monolignol) contained in plant cell wall, whose dehydrogenated polymers (DHP = coniferyl alcohol polymers) are regarded as synthetic lignin, a model of the cell wall. ConA was polymerized by HRP/H_2O_2 in pectin solution in order to mimic the lignification that is the final step of biosynthesis of plant cell wall. The polymerization behaviors and product structures with cluster formation were examined in detail by various physical methods.[505] ConA was also polymerized in the presence of α-cyclodextrin (α-CD) with HRP/H_2O_2 system in phosphate buffer. The presence of α-CD led to DHP with 8-*O*-4′-richer linkages, compared with no additive case. This is probably because of the inclusion complex formation between ConA and α-CD, which suppresses the other linkages like 8−5′ and 8−8′ linkages, because of steric hindrance of the complex (Scheme 79a).[506] A lignin-based macromonomer, lignocatechol (Scheme 79b), was prepared from wood components and oxidatively polymerized by laccase catalyst to produce cross-linked polymers in good yields. Laccase-catalyzed copolymerization of lignophenol with urushiol was also performed to afford the corresponding copolymers in high yields. The thermal stability of these polymers was excellent. The polymerization mechanism was suggested as that the reaction proceeded mainly at the catechol ring through a quinone radical intermediate.[507]

Scheme 80



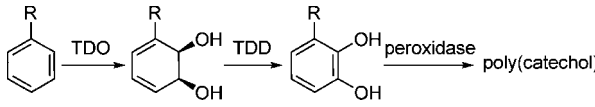
Scheme 81



Oxidative polymerization of 2-naphthol was carried out in a reverse micellar system to give a polymer in single and interconnected microspheres. The polymer showed the fluorescence characteristics of the 2-naphthol chromophore.[508] The polymerization of 8-hydroxyquinoline-5-sulfonate was studied by in situ NMR spectroscopy and the polymerization mechanism was discussed in detail.[509] HRP-catalyzed polymerization of 1,5-dihydronaphthalene (1,5-DHN) produced poly(1,5-DHN) as a dark brown solid consisted of the 1,5-dihydroxynaphthalene unit (A), the 1(or 5)-hydroxy-5(or 1)-oxynaphthalene unit (B), and 1,5-dioxynaphthalene unit (C) (Scheme 80). HRP-catalyzed polymerization of 2,6-DHN proceeded selectively at the aromatic ring to give poly(2,6-DHN) constituted from only the 2,6-dihydroxynaphthalene unit. Reaction of poly(1,5-DHN) with Al(Et)(2-methyl-8-quinolinolato)$_2$ caused Al−O bond formation to give a photoluminescent-aluminum complex. Optical, electrochemical, and thermal properties of these polymers were studied.[510]

Oxidoreductase enzymes contain a catalytic site of a metal (Fe or Cu) moiety. Catalytic functions of the enzymes were mimicked and new enzyme model complexes were created for oxidative polymerizations. Regioselective oxidative polymerization of a phenol compound leading to *2,6-unsubstituted* poly(1,4-oxyphenylene) (PPO) had not been achieved. The regioselective polymerization of 4-phenoxyphenol was first accomplished by using a tyrosinase-model complex (T-MC) having Cu as catalyst to give unsubstituted crystalline PPO having melting points (Scheme 81a).[511−514] The high selectivity is explained; "nucleophilic" $\mu-\eta^2:\eta^2$-peroxo dicopper(II) complex is generated as the sole active oxygen complex from the catalyst and abstracts proton (not hydrogen atom) from the monomer to give phenoxo−copper(II) complex, equivalent to phenoxy radical−copper(I) complex. These intermediates are not 'free' radicals, but 'controlled' radicals, and hence, regioselectivity of the subsequent coupling is regulated. The behaviors in oxidative polymerization of phenol and 4-phenoxyphenol by the tyrosinase-model complex catalyst were well discussed from the

Scheme 82



mechanistic viewpoint.[437,515] The same catalyst induced an oxidative polymerization of 2,5-dimethylphenol, giving rise to crystalline poly(2,5-dimethyl-1,4-oxyphenylene) having melting point of 275−308 °C (Scheme 81b).[516,517]

From *o*- and *m*-cresols, polymers consisting mainly of 1,4-oxyphenylene unit were also formed.[518,519]

Fe-salen (Scheme 77) catalyzed an oxidative polymerization of *p*-*t*-butylphenol and bisphenol A, yielding soluble polyphenols.[520] 2,6-Dimethyl- and 2,6-difluorophenols were polymerized by Fe-salen catalyst to give PPO derivatives. The latter polymer showed crystallinity with melting point higher than 250 °C.[521,522]

Fe-salen catalyzed an oxidative cross-coupling of phenolic polymers onto a phenol-containing cellulose at room temperature under air to produce cellulose-*graft*-phenolic polymers, a cellulose-phenolic polymer hybrid. The reaction was aimed to create an artificial wood polymers, a simple model of lignin-type polymer soluble in a solvent. Actually, the hybrid was soluble in a highly polar sovent like DMF, DMSO, and pyridine.[523]

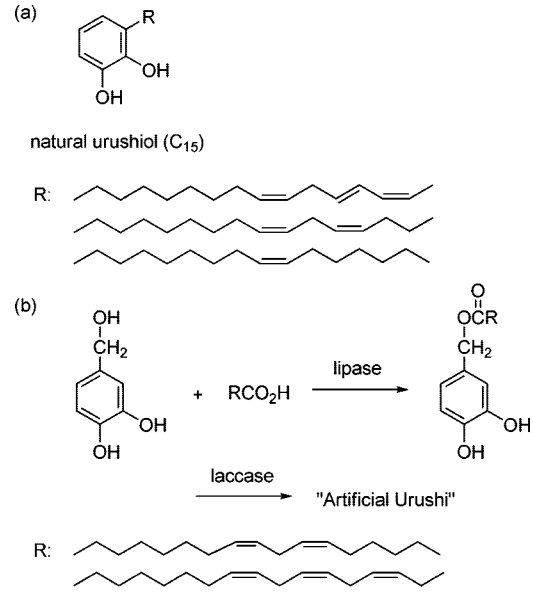
6.1.2. Polymerization of Polyphenols

Here is described the oxidative polymerization of polyphenols, compounds with more than two hydroxy groups on the aromatic ring(s).

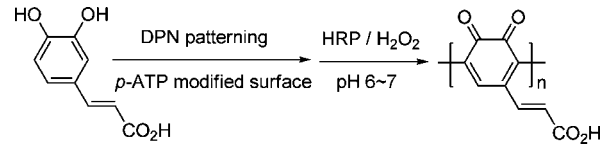
6.1.2.1. Catechol and Its Derivatives. Peroxidase catalyst induces the polymerization of catechol, which is considered to involve an unstable *o*-quinone intermediate to lead to poly(catechol).[524] Laccase also acted as catalyst for catechol polymerization, which was conducted batch-wise in an aqueous acetone gave the polymer with molecular weight less than 1 000.[525] Synthesis of poly(catechol) was demonstrated by the multienzymatic processes (Scheme 82). Aromatic compounds were converted to catechol derivatives by the catalysis of toluene dioxygenase (TDO) and toluene *cis*-dihydrodiol dehydrogenase (TDD), followed by the peroxidase-catalyzed polymerization to give the polymer with molecular weight of several thousands.[526] A biomimetic peroxidase catalyst, iron-porphyrin, was employed for the catechol polymerization, and structures of isomeric dimers products were characterized in detail to discuss the coupling mechanism.[527]

"Urushi" is a Japanese traditional coating material showing excellent toughness and brilliance for a long period. It is a cross-linked coatings of resinous sap "urushiols" obtained from the *Rhus vernicifera* tree. The urushiol consists of a mixture of catechol derivatives having an unsaturated hydrocarbon chain mainly with 1−3 double bonds at 3-position of catechol (Scheme 83a). In nature, the film-forming of urushiols proceeds via laccase catalysis under air. Urushi is a renewable resource and an only example in nature that utilizes enzymatic catalysis for bringing about the practical use.[495,528,529] In vitro enzymatic hardening reaction of the catechol derivatives was induced using laccase as catalyst to afford the cross-linked film showing excellent dynamic viscoelasticity.[530] A fast drying hybrid Urushi was developed; Kurome Urushi was reacted with silane-coupling agents possessing an amino, epoxy or isocynate group, resulting in shorter curing time for Urushi.[531]

Scheme 83



Scheme 84



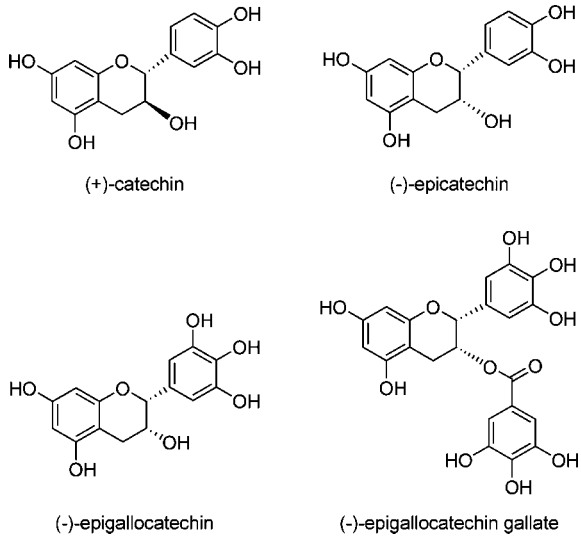
Natural urushi chemistry was mimicked because of the difficulty in chemical synthesis of the natural urushiol. New urushiol analogues were developed for the preparation of "artificial urushi" (Scheme 83b).[492,532,533] The urushiol analogues were cured using laccase catalyst in the presence of acetone powder without using organic solvents, yielding the brilliant film with the high gloss surface (see also the above).

The laccase-catalyzed curing of three natural oriental saps was performed under air (hence O_2 as oxidant) in the 80% humidity at 30 °C and the physical properties of the products were examined in detail.[534] The saps are urushiol from Japan, China, and Korea, laccol from Taiwan and Vietnam, and thitsiol from Thailand and Myanmar, and these saps have little different structures, although all are catechol derivatives. The curing of urushiol and laccol proceeded very fast; the drying time was 2−4.5 h. High values of hardness and Young's modulus were recorded. On the other hand, the drying time of the film of thitsiol was over 24 h. This may be the result of the higher laccase activity for 3-substituted catechol. A hard film was not obtained from thitsiol.

Nanometer scale surface patterning of caffeic acid (3,4-dihydroxycinnamic acid) was achieved on 4-aminothiophenol (*p*-ATP) modified gold surfaces by a dip pen nanolithography (DPN) method. HRP-catalyzed polymerization was applied on the patterned caffeic acid features (Scheme 84).[535] The product polymer did not much contain the C−O−C coupling unit on the surface reaction, which is widely observed in the HRP catalyzed polymerization in solution. *p*-ATP monolayer acted as a template for caffeic acid and the regioselective polymerization was induced to facilitate the exclusive C−C ring coupling because of the participation of a quinone structure from free radical resonance contribution during the reaction.

6.1.2.2. Flavonoid Compounds. Flavonoids are polyphenols having more than two phenolic OH groups on the

Scheme 85



aromatic rings. They are bioactive, found in a variety of plants and used as important components of human and animal diets. Flavonoids are benzo-γ-pyrone derivatives consisting of phenolic and pyrane rings. Flavonoids are usually subdivided according to their substituents into flavanols, flavones, flavanones, chalcones, and anthocyanidines. There has been increasing interest in flavonoids due to their biological and pharmacological activity including antioxidant, anticarcinogenic, probiotic, antimicrobial and anti-inflammatory properties.[442,536] These characteristics are more or less caused by their antioxidant properties. Enzyme-catalyzed oxidative polymerization of flavonoid compounds have been studied mainly in this decade.[440,537]

Green tea contains polyphenols, most of which are flavanols and known as catechins. Catechins in green tea are, typically, (+)-catechin, (−)-epicatechin, (−)-epigallocatechin, (−)-epicatechin gallate, and (−)-epigallocatechin gallate (EGCG) (Scheme 85). Many biological activities are known for green tea; anticancer effects among them are most notable.

Catechin was polymerized via an oxidative coupling in air by plant polyphenol oxidase (PPOx) to give polymers.[538] The polymer properties were similar to those obtained by autoxidation with hydrogen peroxide and to tannins extracted from *Acacia catechu*.[539] The reaction sequence mainly involves formation of unstable *o*-quinone intermediates, followed by their coupling.

The oxidative coupling of catechin by PPOx extracted from grape was examined. In the coupling at pH below 4, colorless products were mostly contained, whereas yellow compounds were formed at the higher pH. Eight dimer fractions corresponding to the major products formed at pH 3 and 6 were isolated. The detailed NMR analysis provided structural hypothesis for five resulting products. The colorless dimers are dehydrodicatechins of the B type with C−O and C−C interflavin linkages. Two yellow dimers correspond to dehydrodicatechin A and to a structure of the quinone-methide type.[540,541]

The coupling of catechin by horseradish peroxidase (HRP) produced oligomers with degree of polymerization (DP) less than 5.[542] Dehydrodicatechin A was isolated and identified from the reaction mixture. Kinetic study on the oxidation of catechin by peroxidase from strawberries showed highly efficient catalytic activity of the enzyme at low concentration

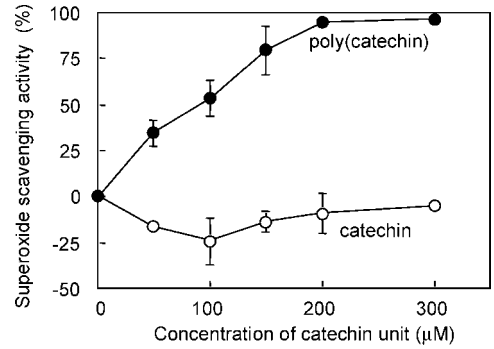


Figure 18. Superoxide anion scavenging activity of catechin monomer and poly(catechin). (Reprinted with permission from ref 550. Copyright 2003 American Chemical Society.)

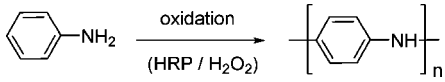
of hydrogen peroxide. The reaction products obtained by HRP catalyst were analyzed by reversed-phase and size-exclusion chromatographies.[543,544] The products obtained by the peroxidase-catalyzed and PPOx-catalyzed oxidative coupling of catechin were comparably studied. Both enzymes produced the similar products.[545] The HRP-catalyzed oxidation polymerization of catechin revealed the formation of two unusual dimers, which were isolated and identified. One is the dicarboxylic acid compound with C−C linkage between C-6′ of B-ring and C-8″ of D-ring. The diacid was formed by ortho cleavage of the E-ring. Another is dimer of C−C linkage between C-2 of C-ring and C-8″ of D ring. In the laccase-catalyzed oxidative coupling, new catechin-hydroquinone adducts were formed.[546]

In the enzymatic polymerization of phenol derivatives, a mixture of hydrophilic organic solvent and buffer is often used as medium for the efficient production of polymers. The HRP-catalyzed polymerization of catechin was carried out in an equivolume mixture of 1,4-dioxane and buffer (pH 7) to give the polymer with molecular weight of 3.0×10^3 in 30% yield.[547]

In the laccase-catalyzed polymerization of catechin, a mixture of acetone and acetate buffer (pH 5) was suitable for the efficient synthesis of soluble poly(catechin) with higher molecular weight around several thousands. The acetone content greatly affected the yield, molecular weight, and solubility of the polymer. The polymer synthesized in 20% acetone showed low solubility toward *N*,*N*-dimethylformamide (DMF), whereas the polymer obtained in the acetone content less than 5% was completely soluble in DMF. In the UV−vis spectrum of poly(catechin) in methanol, a broad peak centered at 370 nm was observed. In alkaline solution, this peak was red-shifted and the peak intensity became larger than that in methanol. In the ESR spectrum of the enzymatically synthesized poly(catechin), a singlet peak at $g = 1.982$ was detected, whereas the catechin monomer possessed no ESR peak.[548] Water-soluble oligo(catechin)s were obtained via HRP-catalyzed polymerization of catechin using a polyelectrolyte like a sulfonated polystyrene as template and a surfactant like sodium dodecylbenzenesulfonate.[549]

The HRP-catalyzed polymerization of catechin greatly enhanced the antioxidant property. For example, the enzymatically prepared poly(catechin) exhibited a much enhanced superoxide anion scavenging activity compared with catechin monomer (Figure 18).[550] Likewise, polyEGCG (Scheme 85) with molecular weight $= 1 \times 10^3$ showed excellent inhibition of xanthine oxidase (XO) about 20 times more amplified than

Scheme 86



the monomer EGCG. Kinetic analysis indicated that poly-EGCG is an uncompetitive inhibitor of XO.[551]

Laccase and tyrosinase from *Ustilago maydis* were used as catalyst for the oxidative polymerization of flavonoid compounds, quercetin and kaempferol. The former produced aggregates with relatively low molecular weight and higher antioxidant activity than the monomer quercetin. The product aggregates from kaempferol reached higher sizes and the antioxidant activity increased in the beginning of the polymerization. Both product polymers showed strong scavenging effect on reactive oxygen species and inhibition of lipoperoxidation.[552]

Other flavonoid compounds were enzymatically polymerized primarily for the enhancement of biological activities such as antioxidant properties. The flavonoids polymerized include quercetin[553] and rutin.[554] The laccase-catalyzed polymerization of a water-soluble rutin derivative, a commercially available product, in an equivolume mixture of methanol and acetate buffer produced the polymer with molecular weight of 1×10^4 in a high yield.

6.2. Oxidative Polymerization of Anilines and Other Aromatic Compounds

6.2.1. Polymerization of Aniline and Its Derivatives

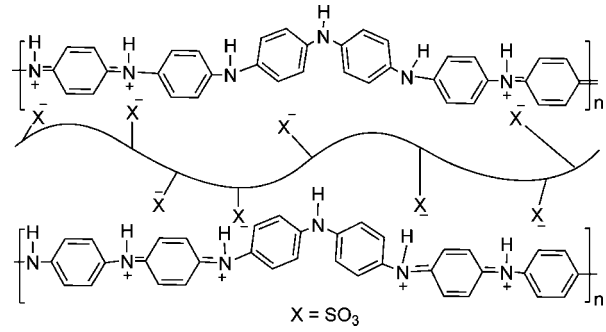
Oxidative polymerization of aniline was conducted a cencuty ago,[555] to give polyaniline (PANI) as aniline black (Scheme 86). In recent years, PANI is one of the most popular conducting polymers because of its stabilities, good electrical and optical properties, which are attractive for technological applications in lightweight batteries, microelectronics, electrochromic displays, light emitting diodes, electromagnetic shielding, sensors and so forth. The well-known methods for the synthesis of PANI are either chemical or electrochemical oxidation polymerization of aniline monomer. The reaction conditions are harsh with extreme pH, high temperature, strong oxidants and highly toxic solvents required. In order to improve the solubility and the processability of the polymeric products, the synthesized PANI is usually postpolymerization-treated with fuming sulfuric acid.

In contrast, enzymatic polymerization of aniline, its derivatives, and other aromatic compounds provides an alternative method of a "green process" toward the formation of soluble and processable conducting polymers. These reactions are usually carried out at room temperature, in aqueous organic solvents at neutral pH. The reaction conditions was greatly improved and the purification process of the final products was simplified when compared to the more traditional methods.[556]

An electroactive PANI film was synthesized by the catalysis of bilirubin oxidase (a copper-containing oxidoreductase). The polymerization of aniline was carried out on the surface of a solid matrix, such as a glass slide, plastic plate, or platinum electrode, to form homogeneous films.[557]

Water-soluble conducting PANI was obtained by HRP-catalyzed polymerization of aniline using H_2O_2 oxidant, assisted with a template in aqueous medium. The aniline complex was formed in the system in the presence of

Scheme 87



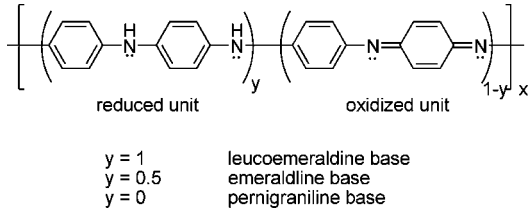
sulfonated polystyrene (SPS), which acted as a polyanionic template. The resulting polymer was complexed to the SPS and exhibited electroactivity. The reduction/oxidation reversibility of the PANI/SPS complex was demonstrated (Scheme 87).[558] The conductivity of the PANI/SPS complex was measured to be 0.005 S/cm. The values increased to 0.15 S/cm after HCl doping and could be increased further with an increase in the aniline to SPS molar ratio. The enzymatic approach is claimed to offer unsurpassed ease of synthesis, processability, stability (electrical and chemical), and environmental compatibility.[559] In the HRP-catalyzed polymerization of aniline, an ionic liquid (IL) was applied to immobilize the enzyme.[560] First, HRP was immobilized by simple solution of 1-butyl-3-methylimidazolium hexafluorophosphate (IL). Then, the HRP/IL mixture was added to the aqueous solution of aniline, dodecylbenzene sulfonic acid and H_2O_2, to give a two-phases reaction system where the HRP/IL droplets are dispersed in the aqueous phase. The polymerization started and ended up with formation of two separate phases consisting from the aqueous PANI phase and the HRP/IL phase, which can be easily separated. The enzyme phase was recycled and reused several times.

In addition to HRP and bilirubin oxidase, laccases were often used as catalyst in the presence of oxygen for the synthesis of PANIs.[561] Also, a biomimetic catalyst like a hydroxy ferriprotoporphyrin compound, hematin, was used for the synthesis of various PANIs. The electrostatic layer-by-layer (ELBL) self-assembly of a polyelectrolyte poly(dimethyl diallylammonium chloride) and hematin was utilized to construct a nanocomposite film catalyst. The conductive PANI was formed not only on the surface of ELBL as a coating, but in the bulk solution.[562,563]

Water-soluble PANIs were synthesized in aqueous solution by enzymatic templating, chemical or electrochemical methods or copolymerization of aniline with sulfonated aniline. The product was electroactive with the average molecular weight of 1.8×10^4. Conductivity was in the semiconducting region (10^{-5} S/cm) at pH 6. The conductivity of poly(2,5-diaminobenzenesulfonate) was about 3−4 magnitude lower because of its branched structure.[564]

In the presence of a template like SPS, enzymatic polymerization of aniline produced PANI having a linear structure, resulting from the electrostatic alignment of the monomer and SPS to promote a para-directed coupling (see Scheme 87). Without the template the PANI structure became branched, which is not desirable for the conducting materials.[565] Conducting property and the structure of PANI have been extensively studied, which gave general explanations as follows. Enzymatically synthesized PANI is normally in the protonated form which can be converted to the unprotonated base form by treatment with aqueous ammonia

Scheme 88



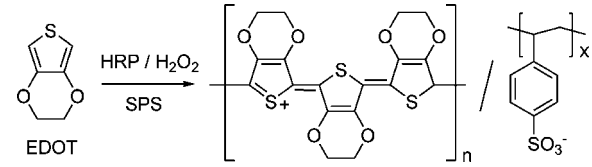
solution or other suitable bases. The unprotonated base form of PANI consists of reduced base units A and oxidized base units B as repeat units, where the oxidation state of the polymer increases with decreasing values of y ($0 \leq y \leq 1$) (Scheme 88). The three extreme possibilities for value of *y* are 1, 0.5 and 0, corresponding to fully reduced PANI (leucoemeraldine), the half oxidized PANI (emeraldine) and fully oxidized PANI (pernigraniline), respectively.[566,567]

HRP-catalyzed oxidative polymerization of aniline was carried out in the presence of a template, poly(acrylic acid) (PAA), and of a chiral compound, 10-camphorsulfonic acid (CSA). The structure of resulting nanocomposites, PAA/PANI/(+)CSA, PAA/PANI/(−)CSA, and PAA/PANI/(±)CSA, was characterized to have a helical conformation induced by the chirality of CSA.[568] A solid state ^{13}C and ^{15}N CP/MAS NMR spectroscopic study on PANIs prepared in various ways was conducted to reveal that the structures were virtually not different as to PANIs obtained from HRP catalysis or from a conventional chemical route.[569] Micellar peroxidase-catalyzed synthesis of chiral PANI was reported to occur by using dodecylbenzenesulfonic acid (DBSA) as surfactant, where HRP and palm tree peroxidase were employed as catalyst combined with hydrogen peroxide. PANI was formed as rice-grain particles with porous surface (average length ~140−180 nm and average width ~70−100 nm). The highest chirality was obtained in the presence of low concentrations of optically active CSA. Unexpectedly, the chiral PANI was produced in the absence of CSA.[570]

The self-doped copolymer of poly(aniline-*co*-3-aminobenzeneboronic acid) [poly(ANI-*co*-AB)], with various mole ratios of two components, was prepared via HRP-catalyzed oxidative copolymerization of both monomers in the presence of an anionic polyelectolyte template (sulfonated polystyrene, SPS) at pH 4.5. The copolymer was used as a boronic acid-based sensor for saccharide molecules. The complexation behaviors between the sensor and a saccharide molecule were examined by UV−vis and IR spectroscopy as well as cyclic voltammetry, and a possible mechanism for the sensitive detection of sugar molecules was proposed.[571] PANI colloid particles were prepared by enzyme-catalyzed oxidative polymerization of aniline in a dispersed media together with toluenesulfonic acid using either poly(vinyl alcohol), poly(*N*-isopropylacrylamide), or chitosan as the steric stabilizer, whereby HRP or soybean peroxidase (SBP) was employed as the enzyme. The colloidal particles are pH- and thermo-sensitive, and were claimed to have potential applications in smart devices such as thermochromic windows, temperature-responsive electrorheological fluids, actuators, and colloids for separation technologies.[572]

In an organic solvent of 1,4-dioxane, HRP catalyzed the enzymatic polymerization of phenylenediamines and aminophenols. Poly(2-aminophenol) and poly(4-aminophenol) resulting from the reactions showed electroactive properties.[573] Poly(azobenzene) and its derivatives has applications in optical devices. A novel polyaniline containing azo groups

Scheme 89



was synthesized by the HRP catalyzed oxidative coupling of 4,4′-diaminoazobenzene. The polymerization was carried out at pH 6.0 in tris buffer with 70% yields. The polymer analyzed by GPC ($M_w = 8.0 \times 10^4$, polydispersity = 4.8) was soluble in DMSO and DMF. Photoexcitation studies indicated that cis−trans isomerization of the chromophore may be the result of structural constraints in the polymer.[574,575]

The dip pen nanolithography (DPN) technology was applied for peroxidase-catalyzed polymerization of 4-aminothiophenol into nanowires on gold surfaces. Reactions were performed in methanol/water (1:1 v/v) with aminothiophenol. The monomer was patterned onto gold by DPN by scratching the surface. Topography of the resulting polymer of aminothiophenol indicates lines 4 mm long with an average width of 210 nm and average height of 25 nm.[576]

HRP-catalyzed polymerization of various aromatic compounds was performed in the presence of metal ions Cu(II), Ni(II), and Fe(III) as imprinting templates. This approach combines molecular imprinting with enzymatic free radical coupling of aniline, tyramine, and phenol. The principle is typically given: first aniline is complexed with Cu^{2+}; HRP and hydrogen peroxide induced the polymerization of the aniline; Cu^{2+} was then taken out from the polymerized products; and then the products were rebonded with Cu^{2+}, in which selectivety for metal ions was desireable.[577]

Peroxidase-catalyzed oxidative polymerization of *o*-phenylenediamine in a mixture of 1,4-dioxane and phosphate buffer produced a soluble polymer consisting of an iminophenylene unit.[578] A new class of polyaromatics was synthesized by peroxidase-catalyzed oxidative copolymerization of phenol derivatives with anilines. In case of a combination of phenol and *o*-phenylenediamine, FT-IR analysis showed the formation of the corresponding copolymer.[579]

6.2.2. Polymerization of Other Aromatic Compounds

For the synthesis of conducting polymer complexes, a biomimetic polymerization of pyrrole and 3,4-ethylenedioxythiophene (EDOT) in the presence of sulfonated polystyrene (SPS) produced polyEDOT/SPS (Scheme 89) and polypyrrole/SPS complexes, where a poly(ethylene glycol)-modified hematin (PEG-hematin) was used to catalyze the polymerization in water at 25 °C at pH 1−2 using H_2O_2 oxidant. The electronic conductivities of the products polyEDOT and polypyrrole were 1×10^{-3} and 1×10^{-4} S/cm, respectively. A copolymer of EDOT and pyrrole was also synthesized to give the copolymer/SPS complex, whose conductivity (0.1−1.0 S/cm) was much higher than that of the homopolymers. The proposed copolymer structure was given as an alternating one; SPS served as a charge-compensating dopant in the complexes to provide properties, such as processability and water solubility.[580]

To expand the application of electronically conducting polyEDOT for biosensing (DNA sensors) and drug delivery system, polyEDOT was synthesized at milder reaction conditions; the synthesis involved very low pH conditions

and oxidants, that are not compatible with biological systems. The problem was overcome with synthesizing polyEDOT by using terthiophene as a radical mediator under milder reaction conditions (pH 3.5−5.5) in the presence of SPS and soybean peroxidase (SBP) as catalyst. The oxidation potential of terthiophene is low enough for initiaton of the polymerization catalyzed by SBP. The oxidized terthiophene helps the subsequent oxidation of EDOT monomer, thus mediating the polymerization. Hence, this oligomer comes into polyEDOT chain. Using conjugate oligomers as redox mediators is a new approach, broadening the types of substrates (thiophenes, pyrroles) to be enzymatically polymerized under benign conditions.[581]

HRP-catalyzed oxidative polymerization of EDOT in aqueous solution containing sulfonated polystyrene (SPS) and H_2O_2 oxidant was reported to give a water-soluble polyEDOT. Essential reaction conditions for the polymerization were considered to conduct the reaction at pH 2 at 4 °C (Scheme 89). Film formation stability was good and the structure was well characterized.[582] A similar HRP-catalyzed polymerization of EDOT gave a two-phase reaction system. The enzyme HRP and EDOT form droplets in the solution to trigger the polymerization in the interface, giving rise to water-soluble conducting polyEDOT. Toward the end of the polymerization, the polymer aqueous phase and the HRP/EDOT phase were separated and the latter phase acted as a biocatalyst, which can be recycled and reused, giving a simpler and easier encapsulation method than the conventional encapsulation of HRP into solid supports.[583]

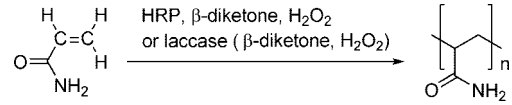
Finally in this section, we note that the green nature of enzyme catalysts has been mentioned in many of papers, giving good examples of green polymer chemistry.

7. Synthesis of Vinyl Polymers

The main target polymers of enzymatic polymerization described in the previous sections are polysaccharides, polyesters, polycarbonates, polyamides, and polyaromatics. Hydrolysis enzymes like cellulase, chitinase, and lipases, which catalyze the bond-cleavage reaction with water in vivo, catalyze the bond-formation reaction effectively to produce various target polymers in vitro.[8,15−19,41] The reaction mode is of polycondensation, ring-opening polymerization, or ring-opening polyaddition. Peroxidases and laccase have shown remarkable utility toward the synthesis of polyaromatics targets in vitro, involving the oxidative coupling of variety of substrates with producing radical intermediate species.[21] The mode of these in vitro reactions is close to that of in vivo metabolic reactions of phenolic compounds. Here, vinyl polymerization by peroxidases or laccase catalysts is described, which proceeds according to a radical polymerization mechanism in most cases.

Peroxidases catalyze the oxidation of compounds such as phenols, aromatic amines and mediator compounds for vinyl polymerization in the presence of hydrogen peroxide, leading to the polymerization, in aqueous, nonaqueous and interfacial systems.[23,462] Prior to 1992, there is a short report showing the formation of low molecular weight vinyl polymers of methyl methacrylate (MMA) when studied in a suspension of *E. coli*.[584] Compared with polyaromatics, vinyl polymerization affords better control of polymer characteristics, as demonstrated with a ternary system (enzyme, oxidant and initiator such as β-diketone). Vinyl monomers investigated for enzymatic polymerization are roughly classified into (meth)acrylic type and styryl type.

Scheme 90



7.1. Polymerization of (Meth)acrylic Monomers

Free radical polymerization of acrylamide (Am) via HRP (an Fe-containing enzyme, section 6)-mediated initiation was carried out in the presence of 2,4-pentanedione at room temperature (Scheme 90). The number-average molecular weight (M_n) and polydispersitiy (M_w/M_n) values of the resulting polyAm ranged from 1.5 to 4.6 × 10^5 and 2.0 to 2.4, respectively.[585] For these polymerizations, combinational usage of enzyme with a reducing substrate of β-diketone such as 2,4-pentanedione was essential. Indeed, the enolic form of 2,4-pentanedione is considered to be a key intermediate for the polymerization.

In contrast, laccase (a Cu-containing enzyme, section 6) from *Pycnoporus coccineus* induced the Am polymerization in water at 50−80 °C under argon even without any additives, such as 2,4-pentanedione, as well as hydrogen peroxide, to produce a high moleculat weight polyAm with M_n reaching 1 × 10^6 with $M_w/M_n \approx 2$ for 4−24 h in up to 81% yields. *N*,*N*-Dimethylacrylamide was also polymerized at 65 °C, giving the polymer of $M_n = 7.3 \times 10^5$ with $M_w/M_n = 2.4$ for 24 h in 70% yields. The polymerization was indicative of free radical mechanism, and an initiating species was supposed to relating with a small amount of oxygen (Scheme 90).[586]

In the HRP-mediated Am polymerization at 25 °C, it was necessary to use the three components, HRP, β-diketone, and H_2O_2, for the polymerization to proceed. The suitability of ten β-diketones as reducing substrates was examined. The keto−enol equilibrium is important for the initiating activity. The suitable β-diketone is a compound without steric hindrance at α and γ positions.[587]

The HRP-mediated Am polymerization was studied on the reaction mechanism. The catalytic cycle of HRP involves two intermediate complexes of E*i* and E*ii* (Figure 19).[588] One molecule of hydrogen peroxide generates two catalytically active forms (E*i* and E*ii*), oxidizing the initiator of 1,3-pentanedione to produce two radicals for the initiation of polymerization. Further, a third compound E*iii* may be formed via HRP degradation by H_2O_2. All these three intermediates showed different UV absorption, indicating

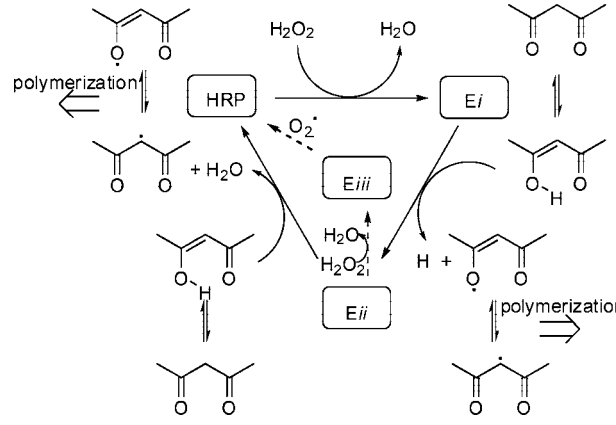


Figure 19. Catalytic cycle of HRP showing the possible mechanism for free radical polymerization of a vinyl monomer with 2,4-pentanedione. E*i*, E*ii*, and E*iii* indicate oxidation states of HRP.

clearly the presence of different oxidation states of HRP depending on the H_2O_2/HRP ratio.[587,589]

The HRP-mediated polymerization of Am was studied on controlling the polymer molecular weight and yield. Molecular weight could be controlled by the initial concentration of HRP and 2,4-pentanedione. These variations were rationalized by using the characteristic expressions of enzymatic catalysis and the classical kinetic equations of free radical polymerization. Further, influences of β-diketone chemical structure on molecular weight[587] were theoretically reconsidered. The results indicated that the influence of the β-diketone structure on the HRP-catalyzed redox system was not a consequence of the chain transfer activity but rather came from their reactivity toward E*i* and E*ii* on HRP-catalyzed radical generation.[590]

The HRP/H_2O_2 catalyst system was extended to a chemoselective polymerization of 2-(4-hydroxyphenyl)ethyl mathacrylate and 2-phenylethyl mathacrylate. The former monomer was oxidatively polymerized at the phenol moiety (section 6) without causing the vinyl polymerization, whereas polymerization of the latter was induced via the vinyl polymerization. A radical initiator of AIBN, however, induced vinyl polymerization of the former monomer; the phenol moiety did not inhibit the radical polymerization.[470]

Enzyme-catalyzed polymerization of MMA using the ternary system (HRP, H_2O_2, and β-diketone) was investigated in water and water-miscible organic cosolvents such as DMF, acetone, dioxane, and THF. In aqueous solution, poly(methyl methacrylate) (PMMA) was obtained from the reaction mixtures of soybeans peroxidase and HRP II (type II) in 48 and 45% yields, respectively. The M_n (M_w/M_n) values of the resulting PMMA were 9.3×10^5 (6.8) and 6.3×10^5 (3.0), respectively. NMR analysis revealed that both enzymes gave PMMA with high syn-diad fractions of 0.85. The syn-diad fractions were also high (0.81−0.87) in a water-organic cosolvent system. Yields of PMMA increased when low dielectric cosolvents like dioxane and THF were used.[591] Polymerizations of Am and sodium acrylate were carried out in aqueous medium with addition of surfactants; anionic bis(2-ethylhexyl)sodium sulfosuccinate (AOT) and cationic cetyltrimethylammonium bromide (CTAB). In aqueous medium, it took 3 h for the complete consumption of Am monomer. The lag-time for chain initiation and propagation reactions was drastically reduced by the addition of AOT or CTAB, and the reaction finished within 60−75 min, giving rise to polyAm with M_n (M_w/M_n) values of 1.4×10^4 (3.2) and 1.3×10^4 (3.2), respectively. In a concentrated oil in water emulsion system, the HRP-mediated Am polymerization finished within 75 min to give PAm with M_w (M_w/M_n) $= 1.1 \times 10^5$ (4.2).[592]

Recently, polyAm was synthesized via HRP-mediated inverse emulsion polymerization of Am in water-in-supercritical carbon dioxide ($scCO_2$) mixture. The most popular emulsions system in $scCO_2$ are those based on ammonium carboxylate perfluoropolyether (PEPE) surfactants. In the PEPE/H_2O/$scCO_2$ system, polyAm, whose M_n value (M_w/M_n) was 4.1×10^5 (3.0), was obtained in a 85% yield from the mixture of HRP, H_2O_2, and 2.4-pentanedione. The polymerization was also performed in the absence of the surfactant, giving rise to a very hard material with high M_n value of 9.1×10^5 ($M_w/M_n = 2.2$). During the polymerization, water was dispersed throughout the $scCO_2$ phase in small droplets by high shear. Formation of inverse emulsion was considered to help the effective polymerization in the absence of PEPE surfactant.[593]

HRP enzyme was immobilized on the thermosensitive hydrogel made from poly(*N*-isopropylacrylamide)-chitosan semi-interpenetrating networks by using glutaraldehyde as bridging agent. The immobilized HRP was then employed as catalyst for acrylamide (Am) polymerization by a redox system (H_2O_2/2,4-pentanedione) in water at room temperature. The product polyAm was obtained in good yields and possessed $M_n = 1.74 \times 10^5$ at the first time use of the catalyst and the activity decreased by the repeated use.[594]

In the HRP-mediated free radical polymerization of vinyl monomers, the use of hydrogen peroxide was needed for the carbon radical generation from β-diketone. When various oxidoreductases, such as laccase, lipoxidase, and sarcosine oxidase, were used as catalyst, radical species was generated in the absence of hydrogen peroxide, and the Am polymerization was induced. For example, laccase from *Myceliophtaora* (ML) or *Pynoporus coccineus* gave polyAm with $M_n = 2.5 \times 10^5$ in 93−98% yields. Methyl methacrylate (MMA) polymerization was induced by ML in the presence or absence of 2,4-pentanedione at 60 °C to give polyMMA of $M_n = 2.4 \times 10^5$ ($M_w/M_n = 1.4$) in 90% yield.[586,595]

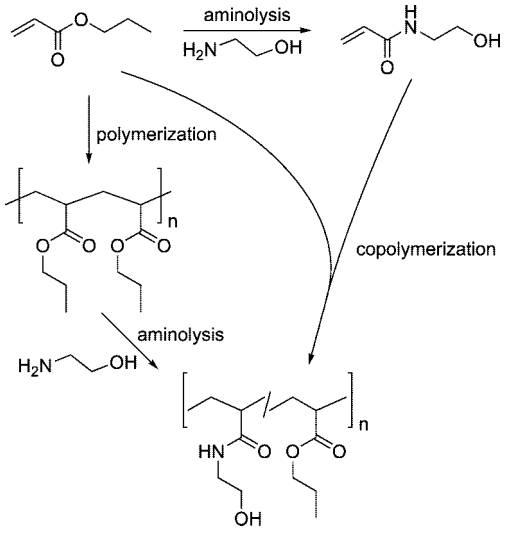
Since the laccase-mediator system (LMS) induces the radical polymerization of vinyl monomers and is environmentally benign, the practical feasibility of Am polymerization using LMS with molecular oxygen as oxidant was examined by conducting the reaction under various reaction conditions. Optimal conditions were slightly acidic reaction media at around 50 °C. The molecular weight of polyAm ($M_n = 6-28 \times 10^4$; $M_w/M_n = 2.5-3.2$) could be controlled via the ratio of monomer to enzyme. Then, it was led to the view that the system did not (yet) fulfill the requirements of economic feasibility on industrial scale, but it was convinced that biocatalytic polymerizations bear a great potential for a greener and more sustainable production of polymers also on industrial scale.[596]

HRP is a ferric heme-containing enzyme and reacts with H_2O_2 to form active species. A biomimetic catalyst of hematin (hydroxy ferriprotoporphyrin) is known to catalyze the polymerization of phenol, and iron in hematin can undergo a cycle of oxido-reductive changes similar to HRP in the presence of hydrogen peroxide. Polymerization of MMA in DMF and Am in Na_2CO_3 buffer was performed by the catalysis, giving rise to the corresponding polymers with $M_n = 1.6-1.8 \times 10^4$, $M_w/M_n = 2.0-2.7$ and $M_n = 1.7-3.7 \times 10^5$, $M_w/M_n = 1.6-2.3$, respectively.[597]

A new polymerization of ethyl acrylate (EA) catalyzed by immobilized *C. antarctica* lipase B (CALB) was reported, with using variable amounts of ethanolamine. The lipase not only catalyzed polymerization of EA but also aminolysis of the pendant ester groups by ethanolamine as nucleophile, giving rise to copolymers of polyEA and poly(*N*-(2-hydroxyethyl)acrylamide). Molecular weight of the resulting polymer was relatively low, with the highest $M_n = 1.9 \times 10^3$, when the feed ratio of ethanolamine/EA was 0.5. A plausible route of copolymer formation is given in Scheme 91; however, the mechanism has not been made clear yet, claiming a new example of enzyme promiscuity.[598]

The Am polymerization was induced from starch main chain, on which radical species was generated by using HRP catalyst, H_2O_2, and 1,4-pentanedione in water and acetate buffer. Thus, the enzymatic polymerization of Am produced

Scheme 91



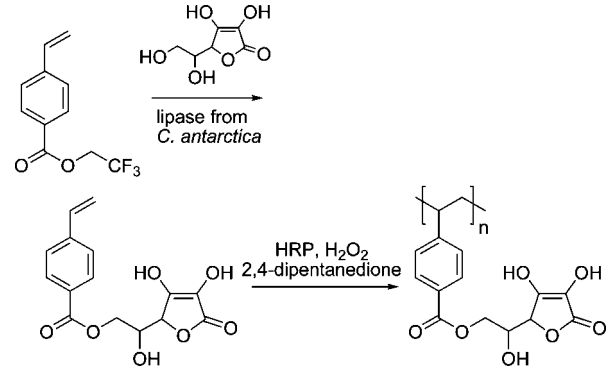
starch-PAm graft copolymers with M_w values of 9.9−30.8 × 10^4 having grafted polyAm content of 5.9−23.6%.[599]

7.2. Polymerization of Styryl Monomers

HRP-mediated radical polymerization of hydrophobic monomers of styrene and its derivatives (4-methylstyrene, 2-vinylnaphthalene) at room temperature was reported.[588] For the efficient progress of the polymerization, selection of the reaction solvent to form a homogeneous solution was very important. The polymerization proceeded involving a radical generated from the initiator effectively, in a mixed solution of H_2O/THF (v/v) = 3, giving rise to polystyrene in 21% yield with M_n and M_w/M_n of 3.2 × 10^4 and 3.1. The styrene polymerization was induced by using five β-diketones (2,4-pentanedione, dibenzoylmethane, benzoylacetone, tetronic acid, and 1,3-cyclopentanedione) and also, 4-hydroxycoumarin as initiator. Polymer yield, M_n and M_w/M_n of the resulting polystyrene were depending on these initiator. For example, yields of polystyrene were raised to 41% and 59% by using tetronic acid and 1,3-cyclopentanedione, respectively. Polymers with higher molecular weight were generated with dibenzoylmethane (M_n = 9.7 × 10^4) and benzoylacetone (M_n = 8.0 × 10^4). Polymerization of 4-methylstyrene and 2-vinylnaphthalene took place similarly.

A major limitation of the HRP-mediated polymerization of styrene is hydrophobic character of styrene monomer and polymer. In an aqueous medium, the insolubility caused the phase separation and/or oligomer precipitation. Then, polymerization of water-soluble vinyl monomer such as sodium styrene sulfonate was achieved in water. As the result, poly(sodium styrene sulfonate) with M_n = 1.6 × 10^5 and M_w/M_n = 3.4 was obtained in 83% yield from the HRP/H_2O_2/2,4-pentanedione reaction system.[600] Poly(sodium styrene sulfonate) was also prepared from the reaction system of hematin/H_2O_2/2,4-pentanedione in aqueous alkaline solvent at pH 11, because hematin shows low solubility at a neutral pH. The reaction using the soluble poly(ethylene glycol) modified hematin, PEG-hematin, proceeded in water at pH 7.0 and after 26 h gave poly(sodium styrene sulfonate) with M_n of 2.2 × 10^5 in 78% yield.[600] The hematin/H_2O_2/2,4-pentanedione system worked in organic solvent such as DMF. Styrene was polymerized at 40−60 °C, giving rise to the poly(styrene) with M_n = 3.0−3.7 × 10^3, M_w/M_n = 1.9−2.4.[597]

Scheme 92



A HRP/H_2O_2/2,4-pentanedione redox catalyst system was applied for a hydrophobic monomer of styrene in a miniemulsion at ambient temperatures with using sodium dodecyl sulfate (SDS) as surfactant. A stable polystyrene latex was obtained with a particle size near 50 nm. The ratio of HRP/H_2O_2/2,4-pentanedione was important to achieve the high conversion of the monomer.[601]

Vitamin C (L-ascorbic acid), an essential nutritive molecule, scavenges harmful radicals such as superoxides, hydroxyls, and singlet oxygen. A strategy was developed to use mild and highly selective enzymatic methods to covalently couple the primary hydroxy groups of ascorbic acid with a vinyl monomer, followed by a second enzymatic reaction catalyzed by HRP to polymerize the vinyl monomer yielding an ascorbic acid-functionalized vinyl polymer (Scheme 92). After the polymerization, the ascorbic acid is present as a pendent group of poly(styrene), and polymers with molecular weight up to 7 000 were formed. The pendent ascorbic acid in polymeric form retained radical scavenging ability against 2,2-diphenyl-1-picryl hydrazyl (DPPH) free radicals. For the complete scavenging of DPPH free radicals (0.2 mM), the polymer at a concentration as low as 238 μM was needed.[602]

Ascorbic acid moiety branched PMMA was synthesized via HRP-mediated vinyl polymerization. At the concentration up to 133 μM, the resulting polymer fully scavenged DPPH free radicals of 0.2 mM.[603] Polymer films prepared from the ascorbic acid functionalized PMMA modulated the proliferation and osteogenic differentiation of early and late-passage bone marrow-derived human mesenchymal stem cells (MSCs) in vitro.[604]

8. Polymer Synthesis via Enzymatic Polymer Modification

Synthesis of new polymeric materials can be achieved not only by the various polymerization reactions but also by modification reactions of the existing polymers. Polymer synthesis via enzymatic modification, however, is so far much less compared with that via enzymatic polymerization as seen in the preceding sections. Enzymes catalyze the modification of a polymer through functional groups located at the polymer terminal, in the main chain or in the side chain in a specific reaction manner, controlling chemo-, regio-, and choro-selectivities, as well as stereochemistry, under mild reaction conditions. The enzyme-catalyzed modification of polymers often provides a green synthesis method of polymer materials. The existing polymers include synthetic polymers, natural biopolymers, half-synthetic polymers, and regenerated polymers. In the polymer synthesis, both the

monomer preparation and the polymer preparation are important processes; however, the latter polymer preparation will be mainly described here, according to the structure of the main polymer to be modified but not to the enzymatic reaction mode or the catalyst enzyme involved. As seen below, enzymatic catalysis for the modification has been adopted for synthesis of polymer conjugates, synthesis of selectively functionalized polymers, cross-linking of polymers, modification of the polymer surface properties, and so forth. Polymer modification is very important; for example, it is well-known that the surface modification of cellulose fibers via hydrolysis by cellulase catalyst is industrially applied.[605]

8.1. Modification of Polysaccharides

8.1.1. Esterification of Polysaccharides

8.1.1.1. Esterification of Cellulose. Site specific chemical modification of hydroxy groups in polysaccharide chains is hardly possible via conventional organic synthetic method. About a preferential acylation of primary over secondary hydroxy groups, for example, the reaction can rarely undergo efficiently with free sugars. In contrast, porcine pancreas lipase (PPL, EC 3.1.1.3) catalyzed a highly regioselective transesterification against various monosaccharides.[606] Further experiments revealed that regioselectivity was controllable by rational choice of enzyme, substrate and solvent.[607−610] Then, preparation of various cellulose-fatty acid esters was performed via immobilized lipase from *Candida Antarctica* (Novozym 435)-catalyzed acylation of cellulose acetate[611] and hydroxypropyl cellulose.[612] For example, Novozym 435 catalyzed the acylation of cellulose acetate (degree of substitution, DS = 2.4) with lauric and oleic acids in acetonitrile. The final conversion of both fatty acids was about 35% after 96 h of incubation at 50 °C. The total ester bonds were increased to 2% after the esterification, suggesting that the acylation took place either in the free hydroxy groups of the cellulose acetate by direct esterification or in the acetyl groups via transesterification.[611] LiCl/dimethylacetamide (DMAc) is a well-known good solvent of cellulose. Acylation of cellulose was progressed effectively in a LiCl/DMAc solution under the homogeneous conditions, and finished within 18 h.[613]

Transesterification of a vinyl ester on cellulose solids was performed in pyridine using protease type VIII (Subtilisin Carlsberg) (EC 3.4.21.62) as an enzyme catalyst.[614] The enzyme is also known to catalyze transesterification of vinyl caproate on amylose.[615] In both reactions, the enzyme catalyzed the regioselective transesterification targeted to the primary C-6 hydroxy groups of glucose units. The cellulose acrylate can be initiated for the graft-copolymerization with acrylonitrile at 16% of grafting yields.[614] Thermal behavior of the resulting polyacrylonitrile-grafted cellulose was different from that of the blend of cellulose and polyacrylonitrile.

These transesterification reactions underwent in organic solvents because the esterification in aqueous medium was considered to be thermodynamically unfavorable.[616] Then, ester formations in both aqueous and nonaqueous mediums were investigated using *Aspegillus niger* lipase-catalyzed acylation of carboxymethyl (CM) cellulose with vinyl acetate. The acylation extent was very low in aqueous buffer; however, it was improved remarkably in the mixed solution of dimethyl sulfoxide (DMSO) and paraformaldehyde. The absence of bulk water and the increased solubility of CM cellulose against the organic solvents were considered to be a main reason for the improvement.[617−619]

8.1.1.2. Lipase-Catalyzed Modification of Other Polysaccharides. Starch (amylose) is a widely utilized polysaccharide of D-glucose mainly linked with α(1→4) glycosidic bonds. Starch is soluble in water or in polar aprotic solvents, where the regioselective acylation is very hard due to the lower catalytic activity of lipase. Hence, starch nanoparticles were incorporated within reverse micelles using the anionic surfactant AOT (sodium bis(2-ethylhexyl)sulfosuccinate). The starch nanoparticles reacted with vinyl stearate, ε-caprolactone and maleic acid at 40 °C in toluene by the catalysis of lipase such as Novozym 435 and SP 525, and gave starch esters with DS of 0.8, 0.6, and 0.4, respectively. Only C-6 position of glucose was reacted in a regioselective manner.[620]

Konjac glucomannan (KGM) is a polysaccharide consisted of β(1→4) linked D-glucose and D-mannose. There are short side branches at the C-3 position of the mannose units, and acetyl groups randomly at C-6. In a solvent free system with 6 mL of vinyl acetate, C-6 of KGM was acylated regioselectively by the catalysis of Novozym 435. The reaction was affected by water content because it plays an important role in enzymatic catalysis with controlling the flexibility of the protein. The optimal water activity, reaction temperature, shaking rate and enzyme dosage were 0.84, 50 °C, 200 rpm, and 400 U/mL, respectively.[621]

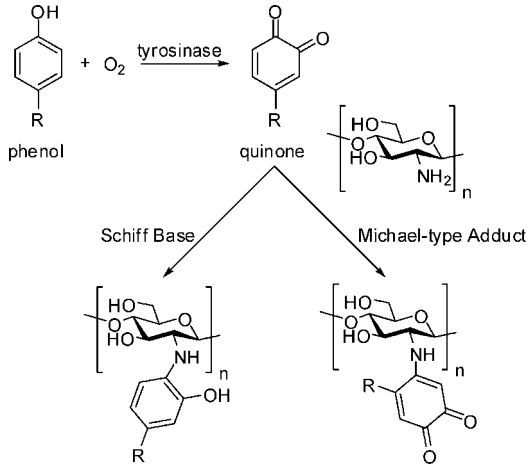
The synthesis of dextrin acrylate with different degrees of substitution ranging from 10% to 70% was reported. Vinyl acrylate was transesterificated to dextrin by the catalysis of Proleather FG-F, a protease from *B. subtilis*.[622] The ammonium persulfate/*N*,*N*,*N*′,*N*′-tetramethylenethylenediamine system initiated a free radical polymerization of dextrin-vinyl acetate in water, and gave dextrin hydrogels. These hydrogels are expected to be utilized as biocompatible and biodegradable scaffold materials for a variety of biomedical applications.

8.1.2. Chitosan Conjugate Formation Catalyzed by Tyrosinase

Mushroom tyrosinases (EC 1.14.18.1) are oxidative enzymes capable of converting low-molecular weight phenols and accessible tyrosyl residues of proteins like gelatin into quinones. These quinones are reactive and undergo nonenzymatic reactions with a variety of nucleophiles. The primary amino groups of chitosan reacted with the quinone, giving rise to chitosan-natural phenol or chitosan−catechin conjugates via a Michael-type addition and/or a Schiff base formation (Scheme 93).[623]

Tyrosinase catalyzed the conjugate-formation reaction between chitosan and various proteins such as cytochrome C,[624] organophosphorus hydrolase (OPH),[624] histidine tagged chloramphenicol acetyltransferase (His-CAT),[624] gelatin,[625,626] green fluorescent protein,[627] silk fibroin,[628−630] and silk sericine.[631] Tyrosinase-catalyzed reactions enabled proteins to be covalently tethered to a three-dimensional chitosan gel network. This covalent coupling permitted the easy fabrication of biocatalytically active hydrogel-based membranes, films, and coating for a wide range of applications. Combining the biodegradable and biocompatible properties of chitosan, these novel hybrid biomaterials are expected to become a promising material for gene and drug delivery, as well as for tissue engineering and regenerative medicine.

Scheme 93



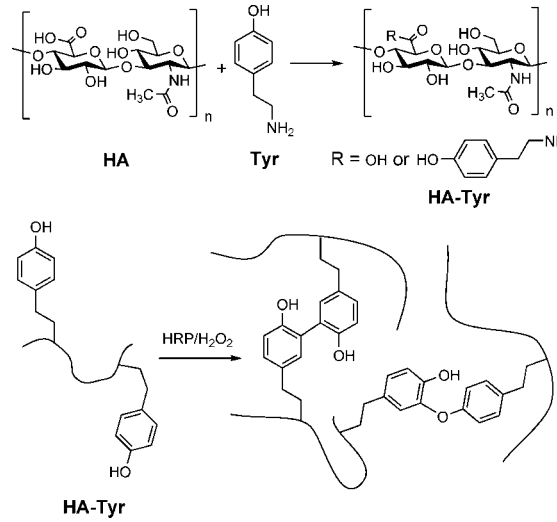
Designed peptides of Kcoil and Ecoil are known to heterodimerize in a highly specific and stable fashion to adopt a coiled-coil structure. Kcoil functionalized chitosan was prepared using the tyrosinase-catalyzed oxidation of a tyrosine-containing Kcoil peptide. From the surface plasmon resonance (SPR) investigation of coil tagged epidermal growth factor (EGF)/Kcoil chitosan interactions, it was found that the Kcoil chitosan conjugate could capture Ecoil tagged EGF via coiled-coil mediated interaction. In this case, the coiled−coil interaction was relatively low, and some improvements are essential for the general usage. This approach seems to provide with multiple added values to chitosan.[632]

8.1.3. Polysaccharide Gel-Formation

Hydrogels have been used extensively for the controlled release of bioactive molecules and encapsulation of cells. For the preparation of hydrogels, enzymatic cross-linking has attracted attentions as a new desirable synthetic route. Horseradish peroxidase (HRP, EC 1.11.1.7) is an enzyme, which catalyzes the coupling of phenol or aniline derivatives with using hydrogen peroxide as oxidant. By HRP-catalyzed cross-linking in the presence of H_2O_2, a hyaluronic acid hydrogel was prepared from hyaluronic acid (HA)-tyramine (Tyr) conjugate (Scheme 94).[633] The gelation with HPP/H_2O_2 was very fast; it took only 20 s at 1.25 unit/mL of HRP and 2.4 mmol/L of H_2O_2. Further, the resulting gel showed degradability by the catalysis of hyaluronidase. From the in vivo experiments using mice, not only the hydrogel formation of HA-Tyr catalyzed by HRP, but also degradation catalyzed by hyaluronidase were observed.[633] This conveniently formed and biocompatible hydrogel system will provide advantages to drug delivery and tissue regeneration by the controlled release of bioactive molecules and/or cells.

Similarly, HRP-catalyzed preparation of polysaccharide gels such as dextran,[634] alginate,[635] carboxymethylcellulose,[636] and chitosan[637] was reported. The resulting gels involve potentials as gel sheet for cellular adhesiveness and proliferation[638] and cell-enclosing capsules.[639] Recently, the effect of phenol group content in polysaccharides on the characteristics of the resulting hydrogels was investigated. As the amount of phenol groups was raised, gelation time was shortened and the hydrogels became more brittle and more hydrophobic. It is known that the water contact angle of the polymer film in the dried state is one of the most important factors for the favorable adsorption of fibronectin and fibroblast cell growth. Indeed, the phenol group content was of importance

Scheme 94



for cellular behaviors on the gel as well as gelation time and mechanical properties of the gels.[640]

8.1.4. Cyclodextrin Production

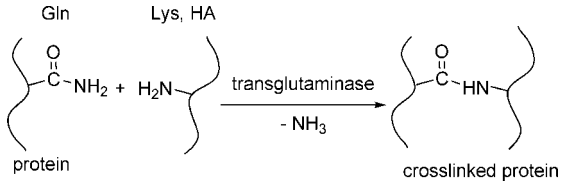
Cyclodextrins (CDs) are cyclic oligomers having mainly 6, 7, or 8 amylose units linked with α(1→4) bond. CDs are widely used in applications like food, pharmacology and chemical industry areas. Starch is degraded by the catalyst of cyclodextrin glycosyltransferase (CGTase, EC 2.4.1.19) to produce cyclodextrins or linear oligosaccharides in good yields, which is a practical method of CDs production.[43]

8.2. Modification of Polyesters

An important synthetic fiber, poly(ethylene terephthalate) (PET), was treated wth an enzyme to improve the surface properties, mainly for better wettability. Two lipolytic enzymes of cutinase from *F. solani pisi* and lipase CA were used for the hydrolysis of the PET films in a buffer solution, in aiming the surface property improvement without damaging the bulk properties. The enzymatic hydrolysis activity for the ester bonds was much affected by the PET crystallinity; relatively high for an amorphous PET and little for a highly crystalline PET sample.[641] To find out a new enzyme for the similar purpose of increasing the hydrophilicity of PET, various microorganisms were screened with using a model compound and some effective enzymes showing esterase activities were found.[642] Laccase was employed for the surface modification of PET, which made the surface properties in an increased hydrophilicity.[643] The enzymatic method requires less amounts of energy and chemicals compared with classical chemical modification employing harsh reaction conditions like a strong alkaline solution.

For the synthesis of biodegradable and amorphous polyketoesters, Novozym 435 was employed as catalyst for end-capping of the terminal OH groups of the polyketoester chain with methacryroyl group, in which vinyl methacrylate was used for the acylation. The end-capped polymer was further cross-linked. Novozym 435 was also used for the starting oligomer synthesis from diethyl ketomalonate, diethylene glycol, diethyl 3-oxoglutarate, and ketoglutaric acid.[644]

Scheme 95



8.3. Modification of Proteins and Poly(amino acid)s

8.3.1. Cross-Linking of Proteins

A protein such as gelatin has been widely employed as a starting material for various gels or conjugates.[623,625,626,645−648] Transglutaminase (E.C. 2.3.2.13, TGase, amine γ-glutaminyl transferase) is known to catalyze transamidation reactions between glutamine residue and lysine residue that leads to the formation of an isopeptide side chain bridge (Scheme 95).[623,625,645−647] TGase was employed as the catalyst for the gel formation from gelatin, from a gelatin−hyaluronan (HA) mixture, and from an HA derivative bearing lysine substituents. The presence of HA in a gelatin gel made the network more hydrophilic and bioactive because of the ability of HA to promote cell regeneration. Such mixed gels are expected to provide good materials to replace flesh tissues.[646] A biocompatible hydrogel was prepared by TGase-catalyzed cross-linking of gelatin from cold fish water skin via enzymatic networking. The rheological and mechanical properties of the hydrogel were examined, which much depended on the degree of the cross-linking.[649]

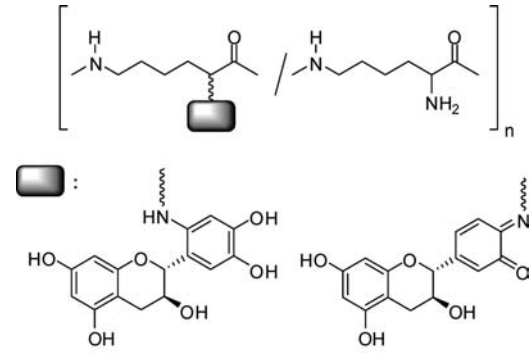
The gel materials from TGase-catalyzed cross-linking of gelatin were characterized in terms of rheologically estimated times, equilibrium swelling in water and in a phosphate buffer, and rigidity modulus.[646] By selection of the substrate sequence structure, 3,4-dihydroxyphenylalanine (DOPA), that confers adhesive strength between the protein and the surface, was incorporated into the synthetic polymer gels for the medical applications. In addition to lysine residue as acyl acceptors, primary alkylamines can be used as substrates, which allowed the selective alkylation of proteins via their accessible glutamine residues.[650]

8.3.2. Oxidative Coupling

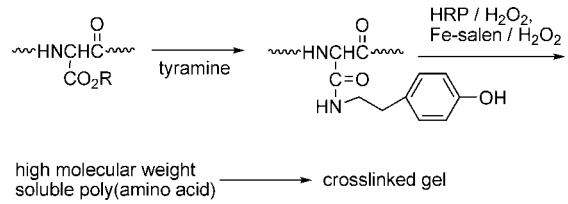
A gelatin−catechin conjugate was prepared by the laccase-catalyzed oxidation of catechin in the presence of gelatin. The conjugate showed a good scavenging activity against superoxide anion radicals and an amplified inhibition effect on human low density lipoprotein (LDL) oxidation.[648] Poly(ε-lysine) is a biopolymer produced from culture filtrates of *Streptomyces albulus* and shows good antimicrobial activity against Gram-positive and negative bacteria; thus widely used as an additive in food industry. A new inhibitor against disease-related enzymes, collagenase, hyaluronidase, and xanthine oxidase, was developed by the conjugation of catechin on poly(ε-lysine) by using laccase as catalyst, in which the conjugation reaction took place between the pendant amino group of poly(ε-lysine) and the catechol ring moiety of catechin (Scheme 96).[651]

An intermolecular oxidative coupling of a phenol moiety of a poly(amino acid) was performed.[652−654] Fe-salen was used as a mimic of HRP; an Fe-salen-catalyzed oxidative coupling of poly(α-tyrosine) did not give a higher molecular weight coupling product, probably because of a steric factor

Scheme 96



Scheme 97



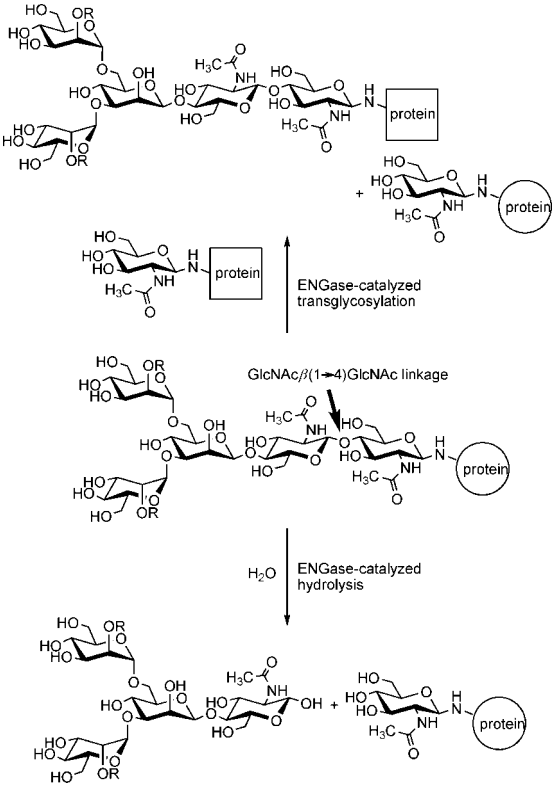
of the phenol moiety. Therefore, tyramine was introduced to poly(glutamine) and poly(asparagine) as a phenol moiety having the spacer (Scheme 97). The tyramine-containing poly(amino acid)s underwent the HRP- or Fe-salen-catalyzed oxidative coupling to produce a high molecular weight poly(amino acid); for example, poly(glutamine) having tyramine moiety (50 unit % for glutamine units) showed M_w value of 2.7×10^6 soluble in DMF. In the Mark−Howink−Sakurada equation ($[\eta] = KM^a$), the a value of the polymer was 0.3−0.4, which is lower than that of a linear polymer (0.6−0.8), suggesting a branched structure of the poly(amino acid) with ultrahigh molecular weight. These behaviors were taken as a polymerization of polyfunctional macromolecules as monomer to afford ultrahigh molecular weight soluble polymers. The further prolonged reactions resulted in gel-formation. Similarly, an ulrtahigh molecular poly(*m*-cresol) was obtained by the Fe-salen-catalyzed oxidative coupling. The molecular weight of the product polymer reached M_w value 6×10^6 without formation of insoluble gels.[655]

8.3.3. Synthesis of Glycoproteins

8.3.3.1. Endo-β-*N*-acetylglucosaminidase Catalyst. Glycosylation is one of the most important posttranslational modifications of proteins. Glycoproteins play important roles in the living system in many fields such as protein folding, intracellular trafficking, intercellular communication, etc. Among various kinds of approaches of glycoprotein synthesis, a chemoenzymatic route using endo-β-*N*-acetylglucosaminidases (ENGases) has been focused in this decade.[656−658]

ENGase is an enzyme, which hydrolyzes GlcNAcβ(1→4)-GlcNAc glycosidic linkage located at the connecting region of *N*-linked glycoprotein. Among them, the enzymes from *Arthrobacter protophormiae* (Endo-A), *Mucor hiemalis* (Endo-M), and *Canorhabditis elegans* (Endo-CE) are known to show transglycosylation activity, and they transfer the oligosaccharide blocks of *N*-linked sugar chains to suitable acceptors having a GlcNAc residue. Various glycoproteins with complex structures were synthesized utilizing ENGases-catalyzed transglycosylation (Scheme 98). For example,

Scheme 98



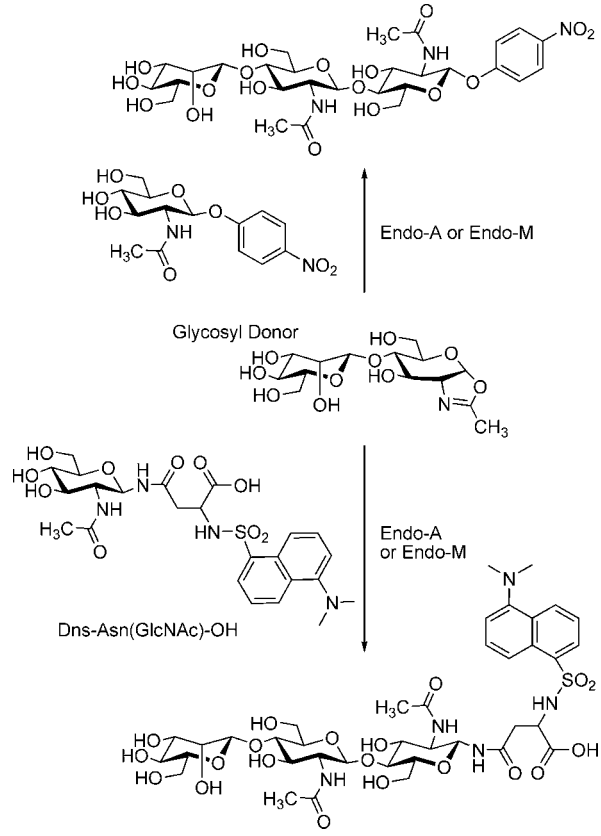
syntheses of eel calcitonin analogue[659] and large HIV-1 envelop glycoprotein fragments[660] were reported. However, their overall yields were generally low, even when an organic solvent was added to the reaction mixture so as to suppress the native hydrolysis reaction of the enzymes.[660,661]

The transition-state of the ENGases-catalyzed reaction is considered to pass through an oxazolinium ion intermediate. Then, transglycosylating activity of endoglycosidases from various origins was examined using a disaccharide donor of a Manβ(1→4)GlcNAc oxazoline derivative and acceptors such as GlcNAcβ1-OpNP and Dns-Asn(GlcNAc)−OH. Core trisaccharide derivatives were synthesized by the catalysis of Endo-A and Endo-M with perfectly controlled regioselectivity and stereochemistry (Scheme 99).[662] These results supported that ENGase-catalyzed transglycosylation proceeded via a substrate assisted mechanism having an oxazolinum ion intermediate in a similar way with family 18 chitinases[36] and family 56 hyaluronidases.[37]

Transglycosylation reactions of Manβ(1→4)GlcNAc and Man_3GlcNAc-oxazoline derivatives to glycosyl acceptors such as a GlcNAc-heptapeptide derived from HIV-1 gp120 and a 34-mer peptide GlcNAc-C34 derived from HIV-1 gp41 were carried out. The reactions took place with high efficiency and the resulting glycoproteins were hardly hydrolyzed by Endo-A. Indeed, oligosaccharide oxazoline derivatives are more reactive substrates for the glycosylation than previously used *N*-glycopeptides glycosyl donors[663] Synthesis of glycopolypeptides carrying two *N*-linked core tri- and pentasaccharides was also achieved.[664]

From the evaluation of the substrate specificity of Endo-A- and Endo-M-catalyzed transglycosylations, a Manβ(1→4)-GlcNAc oxazoline structure is essential for both enzymes. Further, Manβ(1→4)GlcNAc oxazoline derivatives modified at 3-*O*- and/or 6-*O*- positions were recognized for Endo-A as a glycosyl donor.[665,666] At the glycosylation of natural

Scheme 99



series of oxazoline donors with a Manβ(1→4)GlcNAc moiety, Endo-A was more efficient than Endo-M. In case of unnatural series of oxazoline donors containing glucose for mannose substitution, Endo-M showed catalytic activities for various donors. On the other hand, Endo-A was capable of glycosylation with donors having mannose unit at the 3-*O*-position of glucose unit.[667]

Transglycosylation reaction of an unnatural hexasacharide oxazoline to ribonuclease B, which is a small glycoprotein consists of 124 amino acids with a single glycosylation -site at Asn-34, was carried out as a model reaction for the synthesis of *N*-linked glycoprotein.[668] Large oligosaccharide oxazoline derivatives were also used as donor for the transglycosylation to ribonuclease B. A series of glycoproteins with a single glycoform was possible to synthesize. Furthermore, by introduction of an azide group to oligosaccharide oxazoline derivatives, post site specific modification of the resulting glycoproteins using click chemistry was performed.[669]

8.3.3.2. Mutation of Endo-A and Endo-M for Enhanced Glycosylation Activity. To enhance the transglycosylation activity of Endo-M, site-directed mutagenesis on residues at putative catalytic region was investigated. The inherent hydrolytic activity for product hydrolysis was lost by the mutation of Asn-175 residue to Ala (N175A). However, the mutated enzyme showed high transglycosylation activity when sugar oxazoline derivatives were used as donors. Glu-177 works as a proton donor at the hydrolysis reaction catalyzed by Endo-M. On the other hand, an E177A mutant of the enzyme was not capable of promoting transglycosylation even when sugar oxazoline derivatives were used as donors.[670]

Two mutants of Endo-A, E173Q and E173H (E = Glu, Q = Gln, and H = His), were prepared by site-directed

mutagenesis and enzyme-catalyzed reactions of three oxazoline donors were investigated. Glu-173 residue was characterized as the proton donor for catalysis in Endo-A.[671,672] The hydrolysis activity for resulting products was suppressed by both mutageneses. Especially with E173Q, the product hydrolysis was completely abolished. Both mutant enzymes showed transglycosylation activity, giving rise to tetra- and pentasaccharide products from corresponding tri- and tetrasaccharide donors. However, the oxazoline donor was partially hydrolyzed by these mutated enzymes instead of being transferred onto acceptor.[673]

8.3.3.3. Transglutaminase Catalyst. Despite the unnatural structure at the connecting region of the resulting glycoprotein, transglutaminase (TGase, EC 2.3.2.13)-catalyzed transamination reaction is an effective synthetic route for the introduction of a sugar moiety into glutamine (Gln) residue.[674,675] TGase-catalyzed chemoenzymatic synthesis of sugar-displaying protein was examined using mutated insulin as a substrate. Insulin has three Gln residues in the molecule; however, the enzyme did not recognize these Gln residues because of no accessibility, arising from steric hindrance. By the point mutation of N-terminal phenylalanine (Phe) residue of the insulin B chain to Gln residue, transamination reaction proceeded effectively.[676,677]

Additionally, a triadentate glycopeptide was synthesized via site-specific peptide modification of three Gln residues in cyclic polypeptides with sugar moiety. The resulting glycopeptides acted as a blocker of influenza virus hemagglutinin.[678]

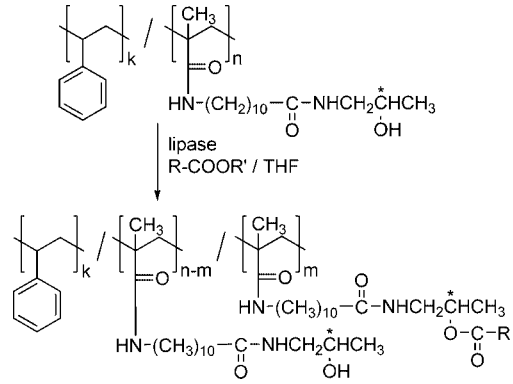
8.4. Modification of Polyaromatics

Lignin is the most abundant biopolymer in nature, next to cellulose. It is a kind of phenolic polymers insoluble in almost all solvents. Laccase- or peroxidase-catalyzed coupling reaction between craft lignin and a water-soluble phenolic compound induced the incorporation of the phenolic compound into the lignin. When the phenol guaiacol sulfonate was incorporated, the craft rignin became water-soluble at pH 2.4, in a low ionic strength due to the introduction of sulfonic acid group.[679] Laccase-induced grafting was described for the synthesis of phenol group-containing polysaccharides like natural lignocellulose-based flax fibers and surfaces with added phenols such as dyed cellulose fibers.[680,681] A phenolic polymer was extracted from *Fucus serratus*, a marine product, and cross-linked oxidatively with catalysis of a vanadium-containing bromoperoxidase (BPO) extracted from a related species *Ascophyllum nodosum*. The cross-linking was initiated by adding the BPO enzyme, KBr, and H_2O_2 to a thin film of the phenolic polymer. A possibility was pointed out, which uses the polymer as a potential component curable in water for a water resistant or coating material.[682]

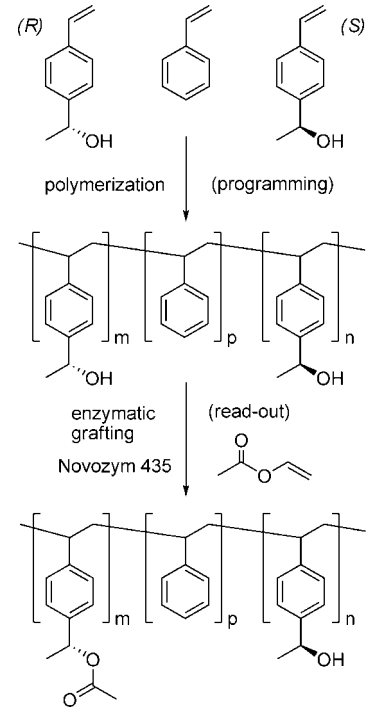
8.5. Modification of Vinyl Polymers and Hydrocarbon Polymers

Regioselective modification of ester functions in poly(methyl acrylate) (PMA) via lipase-catalyzed transesterification was conducted, in which methoxy group was replaced with cinnamyloxy group by the catalysis of the lipase from *Rhizomucor miehei* (lipozyme) in toluene at 50 °C.[683] The transesterification was affected by the PMA chain length; when the degree of polymerization of PMA was in a range of 6−22, the reaction took place in relatively good regioselectivity near the PMA chain ends. The behaviors were interpreted in terms of steric hindrance caused by chain foldings.

Scheme 100



Scheme 101



A lipase-catalyzed acylation of comb-like methacrylate polymers was induced through OH groups in the side chains using the activated esters, vinyl acetate, phenyl acetate, 4-fluorophenyl acetate, and phenyl stearate, as acylating agents. The OH groups in the side chains of the methacrylate/styrene copolymer were acylated in THF at an ambient temperature for 6 days (Scheme 100).[684] Typically, the reaction of the copolymer ($n = 19\%$) with phenyl acetate catalyzed by lipase PF (*Pseudomonas fluorescens*) gave the acetylated copolymer in 40% conversions ($m = 7.6\%$) after 7 days. It is to be noted that the reaction induced an enantioselective acylation; the product copolymer showed an optical rotation of $[\alpha]^{20}_{546}$ of $-1.20°$. After the reaction, the viscosity behavior of the copolymer solution much changed due to the decrease of OH groups, which cause a hydrogen bonding interaction.

A new enzyme-responsive polystyrene derivative was developed, where the chirality was encoded to program the polymer reactivity. As shown in Scheme 101, chiral monomers were copolymerized with styrene to give the chirality-

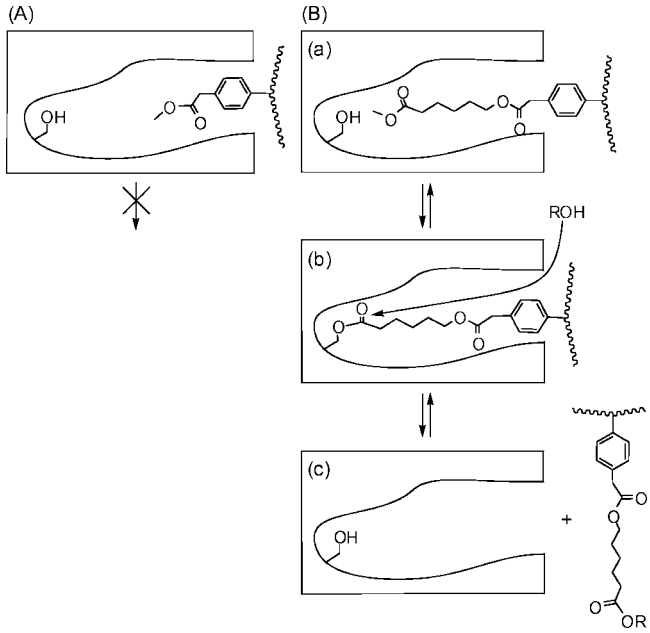


Figure 20. Schematic illustration: (A) Pendant group is too short, and (B) When the pendant group is long (a), the acyl-enzyme intermediate is formed (b), and the transesterification occurred to give the product (c). The active site of the lipase is serine-CH_2OH group.

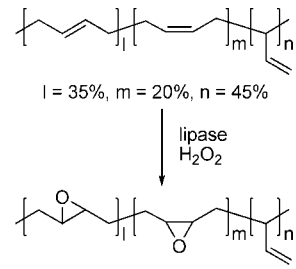
programmed copolymer, which contained about total 45% chiral monomers, with $M_n = 5000-6000$ and polydispersity $= 1.7-2.1$. Novozym 435 was perfectly (*R*)-selective for the acetylation of phenylethanol with vinyl acetate. Therefore, the enzymatic acetylation of the copolymer containing all (*S*)-monomer with vinyl acetate did not take place. In contrast, in a typical case, the acetylation of the copolymer containing 100% (*R*) monomer occurred at 75% of the alcohol groups, which means that the encoded chirality information in the copolymer was accurately read-out (Scheme 101).[685]

Lipase (CALB)-catalyzed modification of pendant ester groups of a polystyrene derivative provided with a clear-cut regioselective transesterification reaction.[363] In the pendant two ester groups, only the ester group distant from the polymer backbone was involved in the reaction. The results suggested that formation of the acyl-enzyme intermediate is sterically not possible for the ester group in the short pendant (A) or the ester group near the backbone, but only for the distant group (B) (Figure 20). This type of enzymatic functionalization of spacer-dependent regioselectivity has not been observed in the chemical reactions.

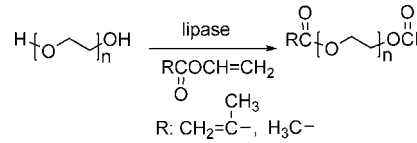
Laccase-catalyzed curing of synthetic vinyl polymers having a phenol moiety in the side chain under air as oxidant was first performed as an application of laccase-catalyzed oxidative polymerization (section 6). An oily sample film of 2-(4-hydroxyphenyl)ethyl methacrylate-containing copolymer with a laccase solution on a glass slide was kept under 80% humidity at 30 °C for 16 h to give the cross-linked polymeric film insoluble in any solvents.[686,687] Poly(4-hydroxystyrene) was modified with aniline with polyphenol oxidase catalyst, but the amount of aniline incorporated was low (1.3%).[688]

Polyacrylonitrile (PAN) fiber was treated with a nitrile hydratase enzyme to convert the pendant nitrile groups to the amide groups; 16% of the nitrile group were reacted to significantly increase the hydrophilicity of the fiber suface.[689]

Scheme 102



Scheme 103



Microbial enzymes were used for the assessment of surface functionalization of PAN and polyamide fibers.[605] It is to be added that currently acrylamide monomer is industrially produced via the nitrile hydratase-catalyzed partial hydrolysis of acryronitrile.

A synthetic polymer of polybutadiene (PBD, $M_n = 1300$, 35% trans, 20% cis, 45% vinyl) was epoxidized with lipase (Novozym 435)-catalyzed oxidation. The reaction was carried out in dichloromethane at 25 °C for 96 h with 10 mol % of acetic acid, 10 wt % of the lipase and 27.5 wt % aqueous solution of H_2O_2 (Scheme 102).[690] The cis and trans alkene bonds of the backbone were epoxidized in yields up to 60%, while the pendent vinyl groups were untouched. This was the first example of an enzyme-catalyzed modification of the backbone of a synthetic polymer, and the method has been often utilized.

Polypropylene (PP) has inert surface; its functionalization was considered difficult but was achieved by using a combination of plasma pretreatment and enzymatic post grafting.[691] First, PP fabric was immersed in an aqueous solution of 2-aminoethyl methacrylate (AEMA) with plasma irradiation; AEMA was considered to connect to the methylene carbon of the PP backbone. Then, plasma-treated PP fabrics were treated with guaiacol sulfonic acid (GSA) in the presence of laccase in phosphate buffer for 2 h at 50 °C, inducing a reaction of the guaiacol moiety with the amino group from AEMA. This gives an example that the enzymatic reaction can be extended to inert polymers such as PP.

Hydroxy-terminated polyisobutylene (a primary alcohol) was methacrylated by using vinyl methacrylate and CALB catalyst in hexane at 50 °C for 24 h. Polyisobutylene alcohols having M_n values of 5240 and 3520 were subjected to the reaction, giving rise to polyisobutylene-methacrylate macromonomers in quantitative conversions.[395] In relation to polyisobutylene polymer, mechanism of enzyme-catalyzed biosynthesis process of polyisoprene (natural rubber, Figure 1) was postulated from the viewpoint of a living carbocationic polymerization process.[692]

8.6. Modification of Polyethers and Polyamines

Poly(ethylene glycol) (PEG) terminals were functionalized via transesterification with vinyl methacrylate or vinyl acetate by *Candida Antarctica* lipase B (CALB, Novozym 435) catalyst (Scheme 103).[394] The M_n values of PEG used were 2000, 4600, and 10 000. In a typical run, vinyl methacrylate (1.2 mmol) was reacted with PEG (0.2 mmol) in THF (4.5

mL) in the presence of CALB (10 mg/mL), with stirring for 24 h at 50 °C. The product telechelics polymer was isolated in 91% conversions and the functinalization at the polymer terminals was quantitative regardless of the molecular weight of PEG.

Polymeric hydrogel biomaterials that are biomimetic both in their synthesis and degradation, were prepared via the site-specific cross-linking reaction of PEG macromonomers with oligopeptide building blocks by using a catalyst of the activated transglutaminase enzyme factor XIIIa. Ligand incorporation into the gels was controlled and living mammalian cells could be encapsulated by the gels. These gels are expected for biomedical applications such as drug delivery systems or smart implants for in situ tissue engineering.[693]

Organosilicon (a polyether) having carboxylic acid group at both ends was end-capped by α,β-ethyl glucoside regioselectively at the primary hydroxy group at C-6 position, which was catalyzed by CALB lipase via an ester bond formation. Thus, a new macromonomer, an organosilicon-sugar conjugate having the glucoside group at both ends, was prepared.[694]

Poly(allylamine) was used for the conjugation of catechin by using laccase catalyst under air. The reaction mode is similar to Scheme 96. The conjugation hardly occurred in the absence of laccase. During the conjugation, the reaction mixture turned brown and a new peak at 430 nm was observed in the UV−vis spectrum. The product poly(allylamine)-catechin conjugate showed a more lasting antioxidant activity against low-density lipoprotein (LDL) peroxidation induced by 2,2′-azobis(2-amidinopropane)dihydrochloride, comparing to the unconjugated catechin.[695] Laccase-catalyzed oxidation of catechin in the presence of amine-containing porous polymer particles gave catechin-immobilizing polymer particles. These polyamine particles showed good scavenging activity toward stable free 1,1-diphenyl-2-picrylhydrazyl radical and 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonate) radical cation. The particles involve a possible application for a packed column systems to remove radical species like reactive oxygen species closely related to various diseases.[696]

9. Concluding Remarks

Enzymatic polymer synthesis has been overviewed for enzymatic polymerization in sections 3−7 and for enzymatic polymer modification in section 8. Almost all the results have been accomplished for these two decades. In some areas, new techniques including enzyme mutagenesis and novel concepts have been created and remarkably advanced recently since the previous Chemical Reviews articles.[8,19] Enzymatic polymerization enabled for the first time the in vitro synthesis of natural and unnatural polymers having a complicated structure, the synthesis of which was otherwise difficult. Such examples may be found in many sections, particularly in the polysaccharide and polyester syntheses.

As described in most above sections and in section 4.3, enzymatic polymer synthesis involves an opportunity for conducting **Green Polymer Chemistry**.[8,17,18,20,22,24,25,143,174,204,363,394−396,482,687] Compared with conventional chemical synthesis, various aspects of enzymatic polymer synthesis are "green".[697] The various aspects are related with problems such as energy-savings, natural resources, environmental desirability, atom-economy, etc. which are seen in more detailed examples below.

- Enzymatic reactions:
  - The reaction rate is normally very large (high turnover number).
  - The reactions proceed with extremely high selectivity; depending on the enzyme, perfect control of regioselectivity, chemo-selectivity, stereochemistry (enantioselectivity) and choro-selectivity.
  - Most of the reactions take place under mild conditions; at a lower reaction temperature, mostly in water or another green solvent, at around neutral pH, under ordinary pressure, etc.
  - The reactions are clean with none or low amounts of byproducts.
  - No need of protection and deprotection chemistry for the substrates.
- Catalyst enzymes:
  - Enzymes are renewable, nontoxic and biodegradable.
  - Enzymes are often free from toxic metals, which is desirable particularly for medical or pharmaceutical applications.
  - Immobilized enzyme catalysts can be readily separated, recovered and recycled for repeated use.
  - They are sufficiently robust to be used in combination with other chemical catalysts, allowing new chemoenzymatic processes.
- Reaction solvents:
  - In addition to water, not only organic solvents but also supercritical fluids,[698] such as carbon dioxide ($scCO_2$) and ionic liquids can be used. Water, $scCO_2$ and ionic liquids are regarded as green solvents.
- Reaction substrates and reagents:
  - Renewable resources (biobased materials) can be often used as starting substrates.
  - Environmentally benign reagents (water, oxygen from air, hydrogen peroxide, carbon dioxide, etc) can be employed.[697]
- Reaction products:
  - Reaction products are biodegradable in most cases.
  - Enzymatic selective reactions often give high value-added products, for example, for a pharmaceutical and/or medical use.

In contrast to the above advantages of enzymatic polymer synthesis, it involves also disadvantages compared with conventional chemical synthesis.

- Conceivable disadvantages:
  - Varieties of substrates and reactions by enzyme catalysts are less than those by chemical catalysts.
  - Availability and the cost of enzymes.
  - Reactions are apparently slow despite high turnover. This is a matter of the amount of catalyst in many cases. (An oversimplified argument: Normally one catalytic active site is contained in one enzyme molecule, whose molecular weight is around 30−50 thousands, being approximately 100 times larger than that of many chemical catalysts. Accordingly, roughly 100 times more amount of the enzyme is necessary to provide the corresponding number of active site of the chemical catalyst.)

Nature is our teacher. We believe it is the definitely right direction to conduct green polymer chemistry with utilizing natural catalysts of enzymes. A hard barrier for the practical utilization of enzymatic polymer synthesis on the industrial scale may be the cost of enzymes. To overcome this problem it is necessary to pursue collaborative works of scientists and

engineers in the field of polymer chemistry, organic chemistry, biochemistry, biotechnology, enzymology, and chemical engineering. Then, it is highly possible to create new enzyme catalysts with much increased activity yet cheaper cost. It is greatly hoped that conducting green polymer chemistry of enzymatic polymer synthesis will contribute to the future sustainable society.

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11. Note Added in Proof

After the submission of this manuscript, papers concerning the present topics have appeared. Some of them are important and to be added here for the update. We would like to thank one of the reviewers for the recommendation to do so.

Section 3.1.2.2: Synthesis of amylose-grafted cellulose was chemoenzymatically achieved.[699] First, maltoheptaose was introduced to the amine-functionalized cellulose, and then the phosphorylase-catalyzed enzymatic polymerization of glucose 1-phosphate (Glc-1-P) initiated from the maltoheptaose to produce amylose-chains which are grafted to the cellulose.[96,97] The amylose graft chain length was tuned as 29−166 glucose units (determined by ^{1}H NMR). The new material, cellulose-*graft*-amylose, was shown to form gels and films.

Section 3.2.2.3: Human hyaluronidase (HAase) is a glycosidic hydrolase enzyme (family 56). The hydrolysis mechanism of hyaluronan (HA) is considered to be caused by a substrate-assisted mechanism involving an oxazolinium intermediate (transition-state) with homology to chitinase. Catalytic activity and reaction mechanism of human HAase have been comprehensively discussed in terms of the active site structure including the acidic and tyrosine residues, point mutation studies of the enzyme, reaction kinetics analysis, etc.[700] The crystal structure data of bee venom HAase recently determined gave much information on the active site structure for human HAase, although bee venom HAase was not an active catalyst for the polymerization to synthesize HA (Scheme 19).[37,146] These results seem very informative to understand a supercatalysis nature of HAase for glycosaminoglycan synthesis.[152]

Section 3.3.1.2: Glycosylation reaction of Glcβ(1→4)-GlcNAc oxazoline (Scheme 24a) catalyzed by *Arthrobacter protophormiae* (Endo-A) was reinvestigated.[701] Under reaction conditions with a normal catalytic amount, Endo-A showed only marginal transglycosylation activity as observed previously. However, interestingly a relatively large amount of Endo-A (e.g., 25 times larger than the normal) showed an enhanced catalytic activity for transglycosylation of the oxazoline as donor to an acceptor, and in the sole reaction of the oxazoline, the ring-opening polyaddition was induced to produce a cellulose−chitin hybrid polysaccharide.[156]

Section 4.3: Lipase has been widely used as catalyst to synthesize polyesters via polycondensation and ring-opening polymerization. Moreover, precision polymer modification using lipase catalyst was emphasized and recent results have been briefly reviewed,[394,395] with stressing the viewpoint of green polymer chemistry using nature's catalysts of enzymes.[702]

Section 8.1.5: Cellulase-Catalyzed Modification of Cellulose Surface (a newly created section). By using a surfactant-enveloped cellulase as catalyst,[54,55] cellulose film surface was lactosylated by lactose in nonaqueous LiCl/DMAc medium.[703] Using the cellulase catalyst came from the fact that both cellulose and lactosyl residue could be substrates for cellulase.[53] Galactose residues of introduced lactose were present on the cellulose film, which was supported by the cell adhesion through the direct interaction between asialoglycoprotein receptors on the rat liver cell surface and the cellulose film surface. This new glycol-modification approach may provide potential application as a bioactive scaffold for cell culture engineering.

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