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Accounts

Enzymatic Polymerization for Precision Polymer Synthesis

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Our recent advances in enzymatic polymerization, defined as chemical polymer syntheses *in vitro* (in test tubes) via non-biosynthetic pathways catalyzed by an isolated enzyme, have been mainly reviewed. The major target macromolecules formed via the enzymatic polymerizations described in this article are polysaccharides, polyesters, and polyaromatics. For synthesis of polysaccharides and polyesters, hydrolases are used as catalysts; hydrolases catalyzing a bond-cleavage reaction by water induce the reverse reaction of hydrolysis, leading to polymer production by a bond-forming reaction *in vitro*. Specific enzyme catalysis provides a novel synthetic route with precise structure control not only for natural biopolymers such as cellulose, xylan, and chitin, but also for unnatural polysaccharides and polyesters, many of which are difficult to be synthesized by conventional methodologies. Oxidoreductases act as catalysts for oxidative polymerization of phenol, aniline, and their derivatives. A new class of phenolic polymers are synthesized without use of toxic formaldehyde under mild reaction conditions. Enzyme-model complexes also catalyze polymerizations for the synthesis of new high-performance polymeric materials.

Enzymatic catalysis for organic synthesis possesses advantages such as high catalytic activity, lack of undesirable side-reactions, operations under mild conditions, and high stereo-, regio-, and chemo-selectivities of reactions in comparison with those of other chemical catalysts. Such characteristics of enzymes have brought about an extraordinarily rapid increase in interest in the area of biotransformations.[1–5]

Production of all naturally occurring polymers is *in vivo* catalyzed by enzymes. Recently, some review reports on *in vitro* synthesis of not only biopolymers but also unnatural synthetic polymers through enzymatic catalysis have been published.[6–15] These enzyme-catalyzed polymerizations receive much attention as examples of a new methodology of polymer syntheses, since in recent years structural variation of synthetic targets on polymers has caused to develop highly selective polymerizations for the increasing demands in the production of various functional polymers in material science.

We define *enzymatic polymerization* as "chemical polymer synthesis *in vitro* (in test tubes) via non-biosynthetic (non-metabolic) pathways catalyzed by an isolated enzyme".[6,7,9,12] Enzymes are generally classified into six groups. Table 1 shows typical polymers produced with catalysis by respective enzymes.[9] The target macromolecules for the enzymatic polymerization have included polysaccharides, poly(amino acid)s, polyesters, polycarbonates, polyaromatics, and vinyl polymers. In many cases, enzymatic polymerization enables the synthesis of polymers, which otherwise are difficult to prepare.[9] Enzymatic polymerization often provides an environmentally benign process, where starting materials and products are within the natural material cycle; this is in the context of "green polymer chemistry".[12–14]

In this account, our recent research topics on synthesis of polysaccharides, polyesters, and polyaromatics by using enzymes and enzyme-model complexes as catalyst are mainly described. As for syntheses of the two former groups, hydrolase enzymes catalyzing a bond-cleavage reaction by water have been used as catalysts. It is generally accepted that a hydrolase-catalyzed reaction is virtually reversible, and hence selection of the reaction conditions is important to control the equilibrium. Based on this concept, we have succeeded in using hydrolases as catalysts for the reverse reaction of hydrolysis, leading to polymer production via a bond-forming reaction. Some oxidoreductases and their model complexes have been found to catalyze an oxidative polymerization of phenol and aniline derivatives to give the functional polyaromatics.

1. Polysaccharides

Carbohydrates are among the most important molecules in living systems, like proteins and nucleic acids. Oligo- and polysaccharides have multiple functions, for example, construction of cell walls, energy storage, cell recognition, and immune response. Such functions result from the complexity of their sequence (regio- and stereochemistry) and high molecular weight. These multifunctional carbohydrates are essential for many fields of research. Therefore, the development of an efficient synthetic methodology has been strongly demanded. Although many synthetic strategies of carbohydrates based on organic synthesis were proposed, perfect control of the regio-

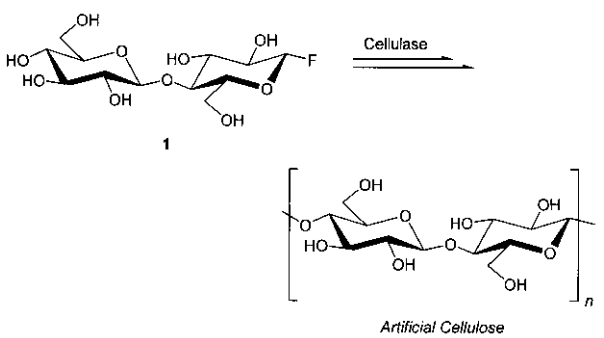
Table 1. Classification of Enzymes and *in Vitro* Production of Typical Polymers Catalyzed by Respective Enzymes

Enzymes	Typical Polymers
Oxidoreductases	Polyphenols, Polyanilines, Vinyl Polymers
Transferases	Polysaccharides, Cyclic Oligosaccharides
Hydrolases	Polysaccharides, Poly(amino acid)s, Polyamides Polyesters, Polycarbonates
Lyases	
Isomerases	
Ligases	Polyesters

and stereochemistry of the glycosylating processes remained as one of the most difficult problems. This section describes some successful results from our recent studies on enzymatic polymerization for polysaccharide synthesis via a nonbiosynthetic path, utilizing advantageous characteristics of glycosidases as catalysts.

1-1. Synthesis of Artificial Cellulose. Cellulose is the most abundant organic resource on the earth; some 10^{11} tons are photosynthesized and biodegraded annually. It is used not only as renewable and biodegradable important structural materials for woods, fiber, and paper, but also as biomaterials for scientific and medical purposes. Since cellulose is a representative of natural polymers and hence a symbolic substance for polymer scientists, Staudinger, Mark and others started its structural studies in the 1920s. Such studies opened the door to polymer science. The most important motivation for us was that the *in vitro* synthesis of cellulose had not been achieved, despite many attempts over the last half a century.[16–19]

For the enzymatic polymerization to occur, the monomer must be recognized by the enzyme as a substrate. The glycosyl donors most frequently employed as substrates for glycosidases are free sugars, phenyl glycoside derivatives and glycosyl fluorides. The glycosyl donors for phosphorylases and glycosyltransferases in the biosynthetic pathway are the sugar 1-phosphates and the sugar nucleotides, respectively. Among these glycosyl donors, the glycosyl fluorides are the most effective substrate for the enzymatic glycosylation because of the following advantages. First, the size of fluorine atom is close to that of oxygen atom and can be acceptable at a catalytic site of the enzyme. Second, due to the excellent nature of fluoride anion as a leaving group, the substrate readily forms an activated glycosyl-enzyme complex by elimination of fluoride anion from anomeric carbon atom. Third, glycosyl fluorides are much more stable as an unprotected form than other glycosyl halides, which is essential for most enzymatic reactions performed in the presence of water.



Scheme 1.

The first successful study of the cellulose synthesis was thus accomplished.[20] Considering the above advantages of glycosyl fluorides, we designed and prepared β-cellobiosyl fluoride (**1**) as the substrate monomer for cellulase, an extracellular hydrolysis enzyme of cellulose. Substrate **1** was thought to be readily recognized by the cellulase from the results of hydrolysis experiments catalyzed by cellulase.[21] The anomeric configuration of the monomer was designed to form a reactive intermediate leading to $\beta(1\rightarrow4)$ linkage via a "double displacement mechanism".[22] In this reaction monomer **1** behaves as both glycosyl donor and glycosyl acceptor for the enzyme. A mixed solvent of acetonitrile and acetate buffer (pH 5.0) (5:1) gave the best result in terms of the yield of "artificial cellulose" (54% yield) (Scheme 1), and the reaction is believed to proceed via an "activated-monomer mechanism".[20]

The mode of this reaction is a polycondensation with the liberation of HF molecules and does not involve protection and deprotection processes of the hydroxyl groups. The artificial cellulose was proven to have a structure similar to that of natural cellulose by CP/MAS ^{13}C-NMR and IR analyses; the regio- and stereoselectivities of the glycosidic bond formation were perfectly regulated to be $\beta(1\rightarrow4)$ linkage. ^{13}C NMR and X-ray

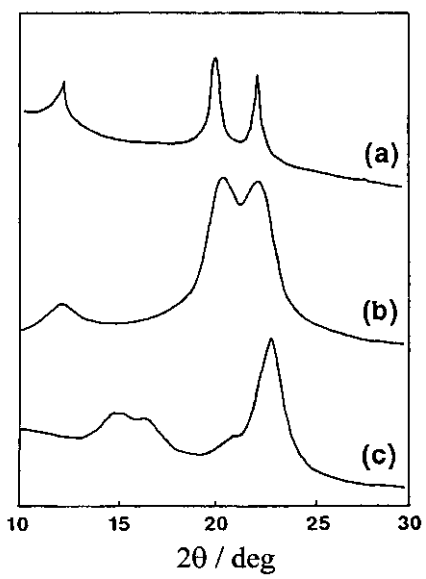
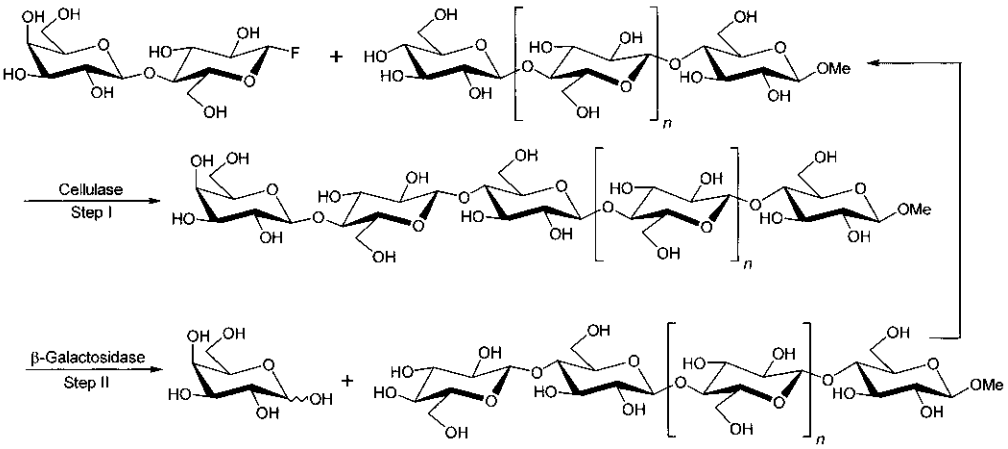


Fig. 1. X-Ray diffractograms of (a) the water-insoluble part of the product (cellulose II), (b) mercerized cellulose (cellulose II), (c) natural cellulose (cellulose I).



Scheme 2.

diffraction studies revealed the crystal structure of cellulose II (Fig. 1).[20]

When a crude enzyme of cellulase was used as catalyst in enzymatic polymerization of **1**, the resultant synthetic cellulose showed the cellulose II allomorph as above. The process of artificial cellulose formation was observed by transmission electron microscopy (TEM), which could be observed as early as 30 seconds after initiation of the polymerization.[23] The electron diffraction pattern of the product was typical for the crystalline structure of cellulose II. Interestingly, when a partially purified cellulase preparation from fungus *Trichoderma viride* was used for the polycondensation of monomer **1** under optimized reaction conditions of a selected mixed solvent system of acetonitrile and acetate buffer, the other allomorph, cellulose I, was produced. This achievement represents the first successful *in vitro* synthesis of cellulose I.[24] Cellulose I allomorph has a highly ordered structure that has long been believed to be produced only in living cells. Cellulose I has a parallel structure of glucan chains known as the thermodynamically metastable form, whereas cellulose II has an antiparallel structure as the more stable form (Fig. 2, see also Illustration). This control of the higher ordered structure during polymerization is, to the best of our knowledge, the first example in polymer synthesis.

The selectivity of these two allomorphs strongly depends on both the purity of the enzyme and the polymerization conditions. For expressing this selectivity, we proposed a new concept "choroselectivity" (Fig. 2).[25]

When the polymer chains have direction, for example, a single cellulose chain has a direction of reducing end to non-reducing end, a parallel or an antiparallel directional relationship is derived due to noncovalent interactions. If the polymer chains assemble to show one preferential direction of the allomorph during polymerization, the reaction is defined as "choroselective". The term "choros" is a Greek word "Χωρος" meaning "space". This concept is, in principle, applicable to all polymerization reactions producing polymer chains having direction.

The above method using a fluoride substrate and another method using a 1-phosphate substrate for enzymatic catalysis led to a new approach to various cellooligosaccharides

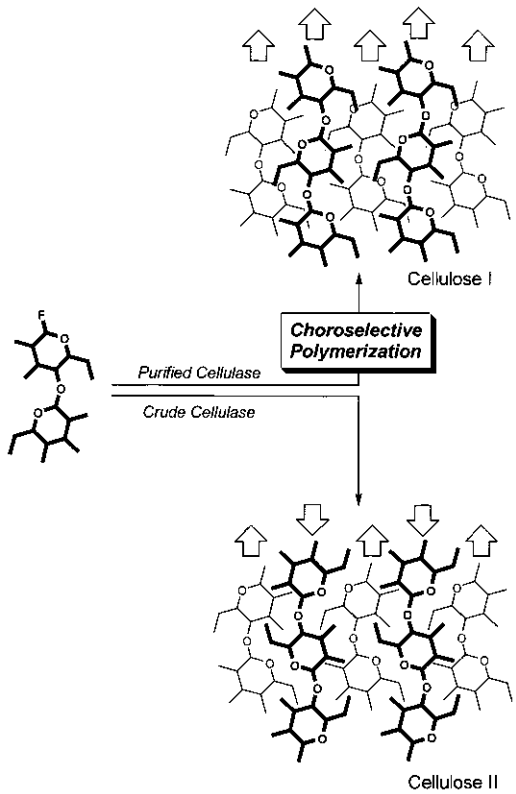


Fig. 2. Crystal structures of cellulose I and II. Enzymatic polymerization of monomer **1** makes it possible to "choroselectively" construct cellulose crystalline allomorph under selected reaction conditions.

(Scheme 2).[26–30]

In our recent study, it was found that enzymatic polymerization of monomer **1** resulted in the formation of artificial cellulose II spherulites, which were consisted of single crystals with the molecular axis oriented perpendicular to the plane. The scanning electron microscopic (SEM) analysis revealed that very thin crystal plates with a thickness of ca. 8 nm originate radially from the center in the spherulites (Fig. 3).[31] This novel spherulite has three-dimensional round shapes. The two-di-

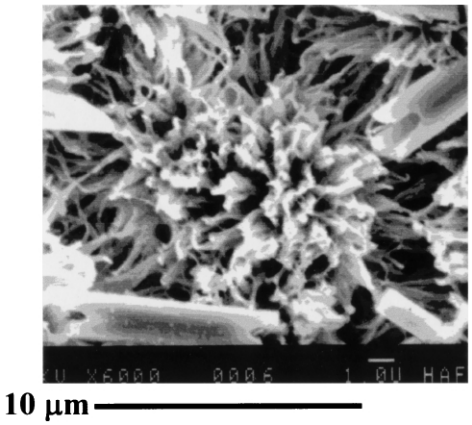


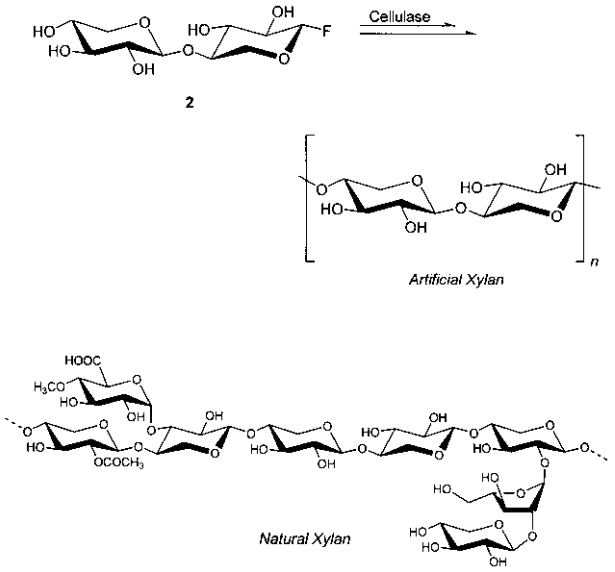
Fig. 3. SEM observation of a plate of artificial cellulose spherulites.

mensional spherulite obtained from bacterial cellulose is completely different.[32,33]

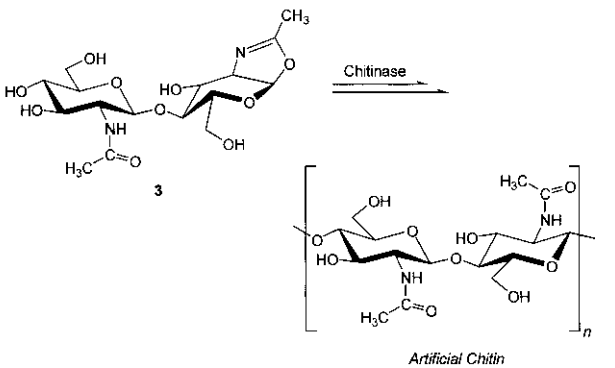
1-2. Synthesis of Artificial Amylose. The similar method using a fluoride substrate monomer was extended to synthesis of amylose, a glucose polymer having $\alpha(1\rightarrow4)$ glycosidic linkage, ie, a polycondensation of α-D-maltosyl fluoride catalyzed by α-amylase produced amylose oligomers.[34] Another method using an α-D-glucosyl phosphate substrate catalyzed by phosphorylase is also a typical example for amylose synthesis and this method was applied to syntheses of graft polymers with amylose side chains.[35–37]

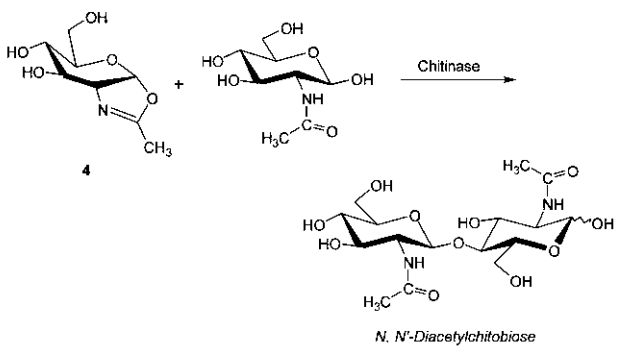
1-3. Synthesis of Artificial Xylan. Xylan, a xylose polymer having $\beta(1\rightarrow4)$ glycosidic linkage in the main chain, is the most important component of hemicellulose in plant cell walls, and an interesting macromolecule because of its supramolecular interaction with cellulose. Naturally occurring xylan usually contains L-arabinose, 4-*O*-methylglucronic acid etc as side chains, its hydroxyl group being often acetylated, and occasionally contains $\beta(1\rightarrow3)$ glycosidic linkage as a minor unit. The first *in vitro* synthesis of xylan has been achieved by an enzymatic polymerization of β-xylobiosyl fluoride (**2**) as a substrate monomer for cellulase.[38] The resulting "artificial xylan" was exclusively composed of $\beta(1\rightarrow4)$ xylopyranose repeating units. Gel permeation chromatographic analysis of its carboxymethylated products showed that the number-average molecular weight is at least 6.7×10^3, which corresponds to a degree of polymerization (DP) of 23 (Scheme 3).

1-4. Synthesis of Artificial Chitin. Chitin, widely found in invertebrates, is a fibrous polymer of $\beta(1\rightarrow4)$ linked 2-acetamido-2-deoxy-D-glucopyranose, the *N*-acetylation being about 20% incomplete. It is the most abundant substance in animals, being estimated as about 1% in quantity for cellulose. It has attracted much interest in numerous scientific and application fields as a multifunctional substance.[39] We found a useful method for chitin production via a nonbiosynthetic path, where the reaction proceeded only in the direction of polymerization, by using both a distorted structured monomer and a hydrolysis enzyme. The reaction is designed on the hypothesis that using a distorted glycosyl substrate close to a transition state structure lowers the activation energy, making glycosylation possible even at pH values where the hydrolysis enzyme does not



Scheme 3.





Scheme 4.

induce hydrolysis reactions. The ring-opening polyaddition of chitobiose oxazoline derivative (**3**), a new monomer with a distorted structure having α-configuration at C1, was efficiently caused by chitinase, giving rise to artificial chitin (Scheme 4).[40,41] CP/MAS ^{13}C-NMR spectroscopic analysis showed the structure of resultant artificial chitin to be $\beta(1\rightarrow4)$ glycosidic linkage, indicating that the polymerization reaction proceeded in a regio- and stereoselective manner between chitobiose units with the inversion of configuration at C1. The molecular

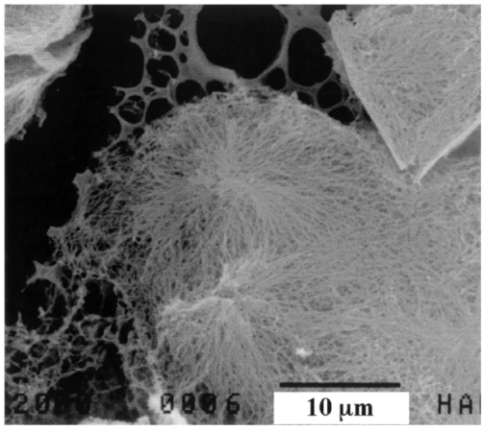


Fig. 4. Electron micrograph of artificial chitin spherulite.

weight of the isolated artificial chitin was determined as Mn > 4×10^3.

The enzymatic polymerization of monomer **3** produced a new type of the artificial α-chitin spherulites.[42] The texture of the artificial chitin was analyzed by optical microscopy in combination with TEM and SEM techniques. Single crystalline ribbons of α-chitin were first formed, followed by the bundlelike assemblies, and the process finally resulted in the formation of spherulites 20–50 μm in diameter (Fig. 4). To our knowledge, this type of high-order molecular assemblies has not been achieved by the other method; the enzymatic polymerization provides a novel route to lead to such abiotic molecular organizations.

The above polymerization of chitin synthesis was applied for a fundamental glycosylation reaction. A reaction of monomeric oxazoline (**4**) as glycosyl donor with *N*-acetyl-D-glucosamine as glycosyl acceptor was catalyzed by chitinase, giving rise to *N*,*N*′-diacetylchitobiose highly selectively (43% yield).[43]

The polymerization mechanism of an oxazoline derivative can be explained as an activated-monomer mechanism. According to our hypothesis, we designed the oxazoline derivative as monomer, which is recognized and protonated at the active site of chitinase from *Bacillus* sp. in the transition state as shown in Fig. 5A.[44,45] The C1 carbon atom of the oxazolinium ion species at the donor site is regioselectively attacked from the β-side by the hydroxyl group at C4 position in the nonreducing end of the propagating chitin molecule at the acceptor site, resulting in formation of $\beta(1\rightarrow4)$ glycosidic linkage, which causes ring-opening of oxazolinium ion. Later, the hydrolysis of chitin by chitinase (*Bacillus* sp.) was reported.[46] The mechanism proposed involves a transition state (Fig. 5C), leading to the hydrolysis product; the transition state is derived from an internal neighboring group participation of the protonated intermediate (Fig. 5B). Thus, a very close structural situation at the donor sites of Fig. 5A and C strongly support our hypothesis that the structure of starting oxazoline derivative monomer is extremely close to the hydrolysis transition state. This structural design enables the monomer to be readily recognized by chitinase due to the already activated substrate monomer and lowers the activation energy of the polymerization; namely, it is required that the transition states of both the

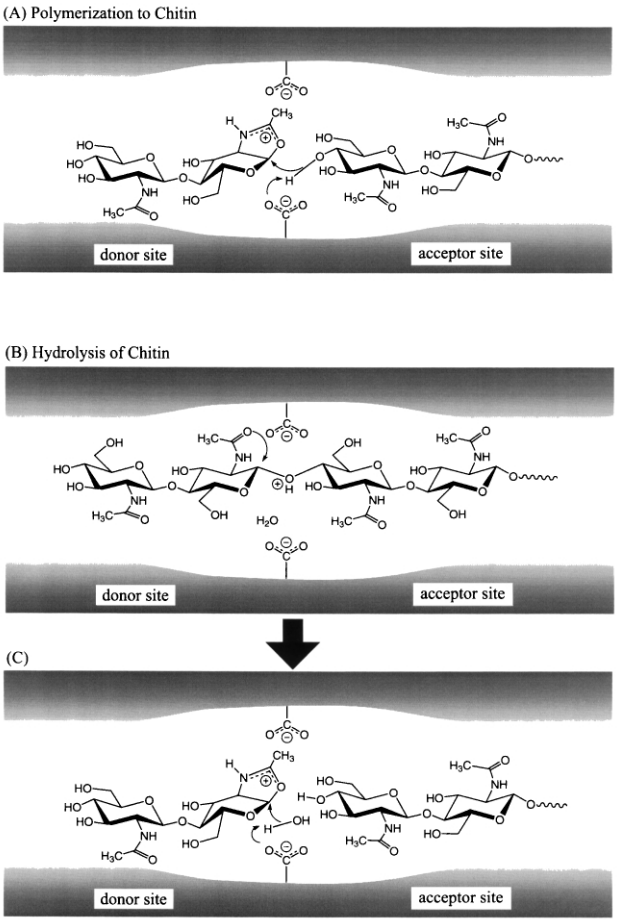


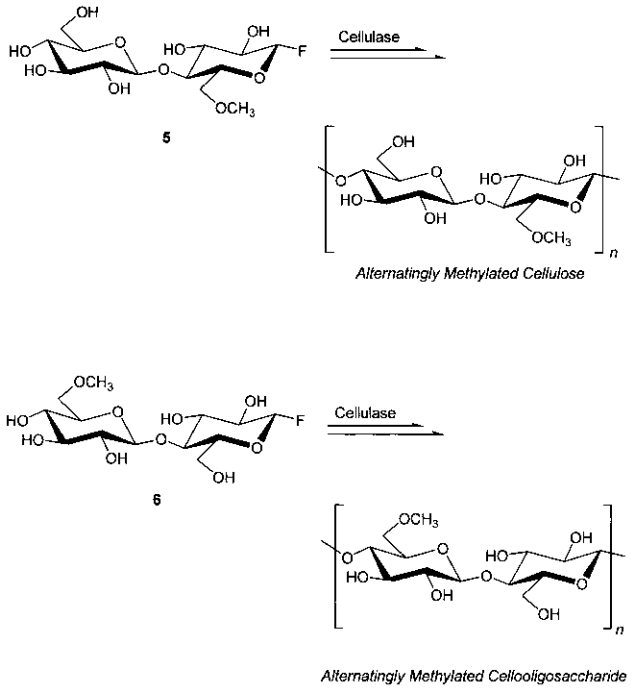
Fig. 5. A postulated transition state for the polymerization of the designed monomer to chitin (A), and proposed transition states for the hydrolysis of chitin by chitinase (B) and (C).

polymerization and the hydrolysis are structurally very common to each other.

We suppose the above hypothesis is always operative in enzyme-catalyzed polymer synthesis, for not only natural polysaccharides described above but also unnatural polysaccharides, various polyesters, and even polyaromatics.

1-5. Synthesis of Alternatingly Methylated Cellulose, an Unnatural Polysaccharide. For the enzyme function Emil Fischer proposed a "Lock and Key" principle in 1894. The principle is still valid; however, the lock and the key do not always absolutely correspond to one-to-one to the enzyme-substrate relationship. There is some allowable range in structural variation of the substrate which can be molecularly recognized by the enzyme.

This situation suggests the possibility of unnatural substance syntheses. 6- and 6′-*O*-Methylated β-cellobiosyl fluorides were designed and synthesized as monomer substrates for an enzymatic polymerization.[47,48] Enzymatic polycondensation of these monomers was demonstrated with the aim of synthesizing regioselectively methylated cellulose derivatives. In the hydrolytic behavior of these monomers, both were hydrolyzed by the action of cellulase to afford the corresponding 1-hydroxyl cellobiose derivatives. These results show that



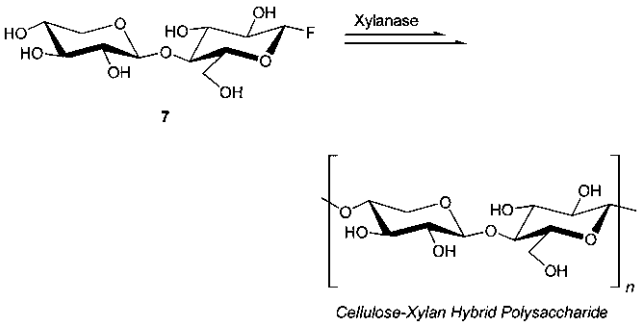
Scheme 5.

the monomers can be recognized as a substrate by the cellulase and are capable of forming an enzyme-substrate intermediate. Enzymatic polymerization of 6-*O*-methylated monomer (**5**) occurred smoothly in an aqueous organic solvent (acetonitrile/acetate buffer) giving rise to a novel cellulose derivative having *O*-methyl groups alternatingly at C6 positions of glucose units. The resulting product showed that the enzymatic glycosylation is proceeded under perfect control of regio- and stereochemistry, leading $\beta(1\rightarrow4)$ glycosidic linkage (Scheme 5). On the other hand, enzymatic polymerization of 6′-*O*-methylated monomer (**6**) gave a mixture of oligosaccharides from which the tetrasaccharide with an alternatingly methylated structure was isolated as the main product.

1-6. Synthesis of Cellulose–Xylan Hybrid Polysaccharide, an Unnatural Polysaccharide. Another example is the construction of hybrid polysaccharides. A new monomer of β-xylopyranosyl-(1→4)-β-glucopyranosyl fluoride (**7**) was designed and synthesized for an enzymatic polymerization catalyzed by xylanase from *Trichoderma viride*. The hydrolytic behavior of monomer **7** catalyzed by xylanase in an acetate buffer (pH 5.0) indicated that **7** is smoothly recognized and hydrolyzed by the xylanase (Fig. 6).

Enzymatic polymerization of substrate monomer **7** occurred smoothly in a mixed solvent of acetonitrile and acetate buffer (pH 5.0) (5:1), giving rise to a novel cellulose-xylan hybrid polysaccharide (Scheme 6). The ^{13}C-NMR spectrum of the resulting product indicated that the enzymatic glycosylation proceeded under perfect control of regio- and stereochemistry, leading to $\beta(1\rightarrow4)$ glycosidic linkage.[49]

Enzymatic polymerization was found useful to control not only the regio- and stereoselectivities but also the suprastructure of the resulting polysaccharides; the first *in vitro* synthesis of cellulose I (choroselective polymerization) is a typical example.[24] Therefore, the enzyme-catalyzed synthesis of oligo-



Scheme 6.

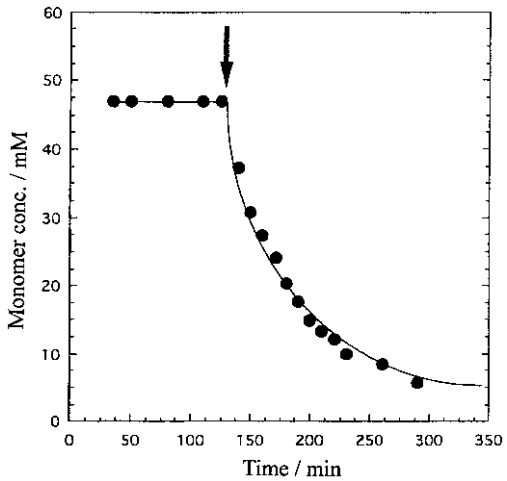


Fig. 6. Xylanase-catalyzed hydrolysis of monomer **7** in acetate buffer (pH 5.0). The arrow shows the time for addition of xylanase.

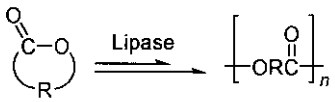
and polysaccharides is a very attractive method because one can construct well-defined oligo- and polysaccharides without complicated protection-deprotection processes of the hydroxyl groups.

2. Polyesters

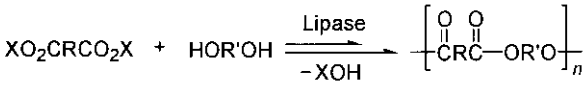
There have been many works on syntheses of aliphatic polyesters by fermentation and chemical processes to achieve biodegradable materials. Recently, lipase-catalyzed synthesis of aliphatic polyesters has been established as another approach to biodegradable polymer production. Lipase is an enzyme that catalyzes the hydrolysis of fatty acid esters normally in an aqueous environment in living systems. However, lipases are sometimes stable in organic solvents and can be used as catalysts for esterifications and transesterifications.[3,4] Polyester syntheses have been achieved by various polymerization modes by utilizing such catalytic specificities of lipase. Three typical reaction types of lipase-catalyzed polymerization leading to polyesters are given in Scheme 7.

2-1. Ring-Opening Polymerization of Cyclic Esters. Various cyclic esters have been subjected to lipase-catalyzed ring-opening polymerization (Fig. 7).[50,51] Lipase catalyzed the ring-opening polymerization of 4 to 17-membered non-substituted lactones (Scheme 8).[52–54] In 1993, we and Gutman's group first demonstrated that medium-size lactones, δ-valero-

Ring-Opening Polymerization of Lactones

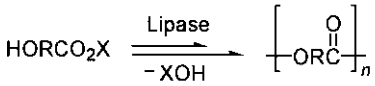


Polycondensation of Dicarboxylic Acids or Their Derivatives with Glycols



X: H, Alkyl, Halogenated Alkyl, Vinyl, etc

Polycondensation of Oxyacids or Their Esters



X: H, Alkyl, Halogenated Alkyl, Vinyl, etc

Scheme 7.

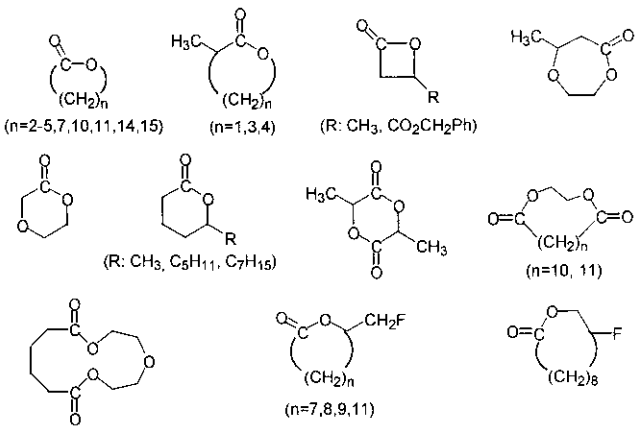
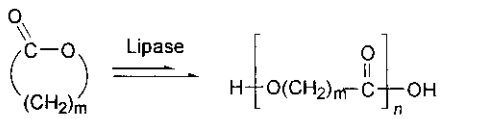


Fig. 7. Cyclic monomers providing polyesters via lipase catalysis.



m=2 (4-Membered) : β-PL m=10 (12-Membered) : UDL
m=4 (6-Membered) : δ-VL m=11 (13-Membered) : DDL
m=5 (7-Membered) : ε-CL m=14 (16-Membered) : PDL
m=7 (9-Membered) : 8-OL m=15 (17-Membered) : HDL

Scheme 8.

lactone (δ-VL, 6-membered) and ε-caprolactone (ε-CL, 7-membered), were polymerized by *Candida cylindracea* lipase (lipase CC), *Pseudomonas cepacia* lipase (lipase PC), *Pseudomonas fluorescens* lipase (lipase PF), and porcine pancreas lipase (PPL),[55,56] which are powdery and commercially available crude enzymes. The terminal structure of the polymer obtained in bulk was alcohol at one end and carboxylic acid at the other. In the polymerization without the enzyme or using the deactivated enzyme, which was prepared by thermal treatment at 100 °C in water, all the monomers were recovered

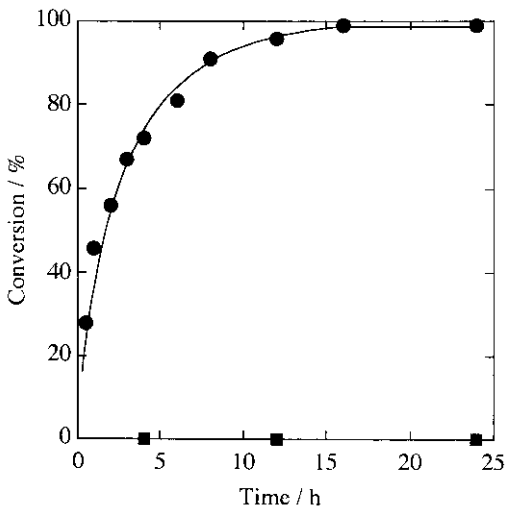
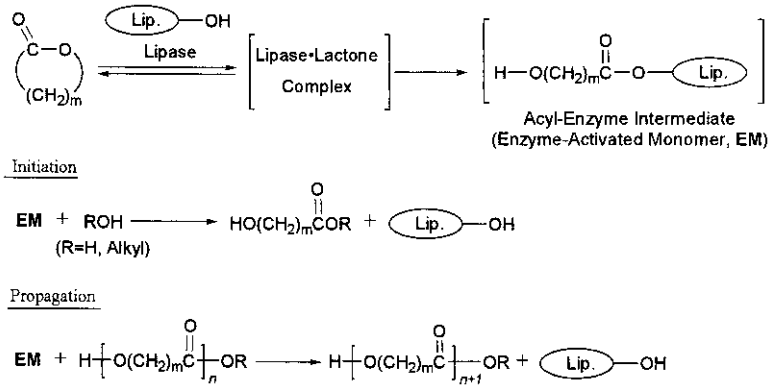


Fig. 8. Time-conversion curves in the enzymatic polymerization of ε-CL using lipase catalysts: (●) lipase CA; (■) lipase PF. The polymerization of ε-CL (1.0 mmol) was carried out using lipase catalyst (10 mg) in bulk at 60 °C under argon.

unreacted, indicating that the polymerization proceeded through the lipase catalysis.[57] In the polymerization of δ-VL catalyzed by these enzymes, the molecular weight of the polymer was relatively low (less than 2000). In case of the lipase-catalyzed polymerization of ε-CL, the molecular weight depended on the lipase origin; the polymerization using lipase PF catalyst at 75 °C produced the polymer with molecular weight of more than 1×10^4, whereas the molecular weight was in the range of several thousands in using lipase CC or PPL catalyst under the similar reaction conditions. The polymerization of δ-VL catalyzed by lipase PF proceeded faster than that of ε-CL.

In the polymerization of lactones by these lipases, the catalyst amount was relatively large, often 20–50 weight% for the monomers, which is due to the slow polymerization rate. We have first demonstrated efficient catalysis of *Candida antarcti-*



Scheme 9.

ca lipase immobilized on macroporous acrylic resins (lipase CA, tradename: Novozym® 435);[58] a small amount of this enzyme (less than 1%) induced the polymerization of ε-CL and the polymerization rate using lipase CA was much larger than that by lipase PF under the same reaction conditions (Fig. 8). These data clearly indicate the high catalytic activity of lipase CA. Furthermore, lipase CA could be repeatedly used for the polymerization of ε-CL. In the range of 5 cycles, the polymerization results hardly changed at all.[57] The reaction rate and molecular weight of the polymer were improved by addition of a small amount of a solvent (toluene).[59] Under appropriate reaction conditions, the molecular weight of poly(ε-CL) reached more than 4×10^4. The reaction medium also affected the polymerization behaviors.[57,59] Solvents having log P values (P: partition coefficient of a given solvent between 1-octanol and water) from -1.1 to 0.49 showed low propagation rates, on the other hand, solvents with log P values from 1.9 to 4.5 efficiently induced the polymerization, leading to high molecular weight polymers.

Lipase-catalyzed reactions are considered to proceed via an acyl-enzyme intermediate. The enzymatic polymerization of lactones is explained by considering the following reactions as the principal reaction course (Scheme 9).[51–53,60] The catalytic site of lipase is known to be a serine-residue. The key step is the reaction of lactone with lipase involving the ring-opening of the lactone to give an acyl-enzyme intermediate (enzyme-activated monomer, EM). The initiation is a nucleophilic attack of water, which is probably contained in the enzyme, onto the acyl carbon of the intermediate to produce ω-hydroxy carboxylic acid ($n = 1$), the shortest propagating species. In the propagation stage, the intermediate is nucleophilically attacked by the terminal hydroxy group of a propagating polymer to produce a one-unit-more elongated polymer chain. The kinetics of the polymerization showed that the rate-determining step of the over-all polymerization is the formation of the enzyme-activated monomer. Therefore, the polymerization probably proceeds via an "activated-monomer mechanism".

β-Propiolactone (4-membered, β-PL) was polymerized by *Pseudomonas* family lipases as catalyst in bulk, yielding a mixture of linear and cyclic oligomers with molecular weights of several hundreds.[61] The lipase-catalyzed polymerization of other 4-membered lactones was reported.[62–64] 9-Membered lactone (8-octanolide, 8-OL) was also polymerized by lipase catalyst.[65] The polymerization of 8-OL catalyzed by lipase PC at 75 °C for 10 days produced a polymer with a molecular weight of 1.6×10^4. As for macrolides, 11-undecanolide (12-membered, UDL),[60,66] 12-dodecanolide (13-membered, DDL),[66,67] 15-pentadecanolide (16-membered, PDL),[60,66,68–70] and 16-hexadecanolide (17-membered, HDL),[71] were enzymatically polymerized. The polyesters with molecular weights higher than 1×10^4 were obtained under appropriate reaction conditions. The lipase-catalyzed polymerization of macrolides (UDL, DDL, and PDL) proceeded even in an aqueous medium.[72]

The reactivity of cyclic compounds generally depends on the ring-size; small- and medium-size lactones show higher reactivity toward the ring-opening polymerization owing to their larger strain in ring in comparison with macrolides (larger sized). Table 2 summarizes dipole moment data and reactivities of unsubstituted lactones with different ring-sizes. The dipole moment value of the monomers is taken as an indication of their ring strain. The medium-size lactones possessed a larger dipole moment due to their larger ring-strain than the macrolides and an acyclic fatty acid ester (butyl hexanoate). The rate constants of the macrolides in alkaline hydrolysis and anionic polymerization are much smaller than those of ε-CL. These data imply that the macrolides have much lower ring strain, and hence show less anionic reactivity and polymerizability than ε-CL.

Figure 9 shows time-conversion curves in the polymerization of lactones using lipase PF catalyst in the presence of 1-octanol. The larger the ring size of the monomer, the larger the polymerization rate. Such behavior is opposite to that by alkaline hydrolysis or anionic polymerization. Thus, Michaelis-Menten kinetics of the polymerization for the quantitative evaluation of enzymatic polymerizability have been performed by using lipase PF as catalyst.[52–54,71,74]

The polymerization followed Michaelis–Menten kinetics: linearity was observed for all the monomers in the Hanes–Woolf plot. $K_{m(lactone)}$ values were not so much different with each other, on the other hand, $V_{max(lactone)}$ and $V_{max(lactone)}/K_{m(lactone)}$ increased as the ring size increased, indicating that the macrolides possess the larger enzymatic polymerizability than ε-CL. Such larger polymerizability is mainly due to the larger reaction rate (V_{max}), but is not due to the binding abilities (Table 2). These results suggest that the reaction process of the lipase-lactone complex to the acyl-enzyme intermediate is the key step in the lipase-catalyzed polymerization; the macrolide

Table 2. Dipole Moments and Reactivities of Unsubstituted Lactones

Lactone	Dipole Moment	Rate Constant		Michaelis–Menten Kinetics[c]		
		Alkaline Hydrolysis[a]	Propagation[b]	$K_{m(lactone)}$	$V_{max(lactone)}$	$V_{max(lactone)}/K_{m(lactone)}$
	Cm	10^4 Lmol^{-1} s^{-1}	10^3 s^{-1}	mol L^{-1}	10^2 mol L^{-1} h^{-1}	10^2 h^{-1}
δ-VL	4.22	55000	—	—	—	—
ε-CL	4.45	2550	120	0.61	0.66	1.1
UDL	1.86	3.3	2.2	0.58	0.78	1.4
DDL	1.86	6.0	15	1.1	2.3	2.1
PDL	1.86	6.5	—	0.80	6.5	8.1
HDL	—	—	—	0.63	7.2	11
Butyl hexanoate	1.75	8.4	—	—	—	—

a) Alkaline: NaOH. Measured in 1,4-dioxane/water (60/40 vol%) at 0 °C. b) Measured using NaOMe initiator (0.06 mol amount) in THF at 0 °C. c) Kinetics of the polymerization was carried out using lipase PF (200 mg) as catalyst in the presence of 1-octanol (0.03 molL^{-1}) in diisopropyl ether (10 mL) at 60 °C.

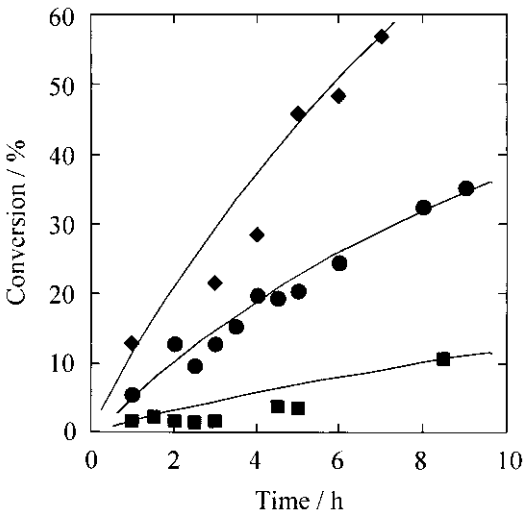


Fig. 9. Time-conversion curves in the enzymatic polymerization of lactones with different ring-sizes using lipase PF catalyst: (■) ε-CL; (●) DDL; (◆) PDL. The polymerization of lactone (0.30 mol L^{-1}) was carried out using lipase PF catalyst (200 mg) in the presence of 1-octanol (0.030 mol L^{-1}) in isopropyl ether (10 mL) at 60 °C.

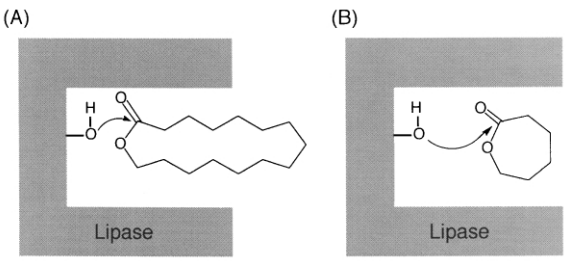


Fig. 10. A possible explanation by model; in the lipase-lactone complex, ring-opening is geometrically (A) favored for a macrolide (PDL) and (B) less-favored for ε-CL.

may be near the serine residue of lipase, favored to open the ring (Fig. 10).

We have examined lipase-catalyzed ring-opening copolymerization of lactones. In the lipase PF-catalyzed copolymerization of δ-VL and ε-CL, a copolymer having a random structure of both units was obtained.[74] The copolymerization of 8-OL with ε-CL or DDL also produced random copolyesters.[65] The formation of the random copolymers in spite of the different enzymatic polymerizability of these lactones suggests the frequent occurrence of transesterification during the copolymerization. By utilizing this specific lipase catalysis, researchers synthesized random ester copolymers (Fig. 11) by the lipase-catalyzed polymerization of lactones in the presence of poly(ε-CL) (Scheme 10).[75,76] Furthermore, we have first demonstrated that the intermolecular transesterification between two different polyesters, poly(ε-CL) and poly(PDL), occurred via lipase catalysis to give the ester copolymer.[75,76]

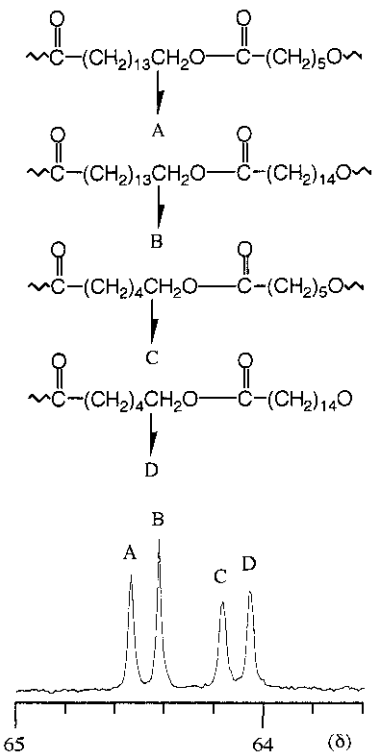
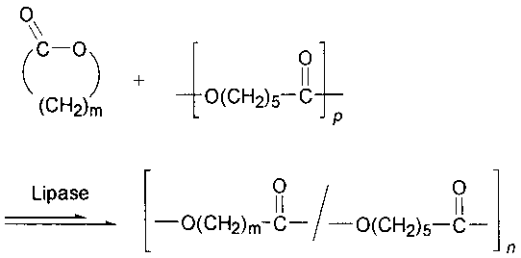


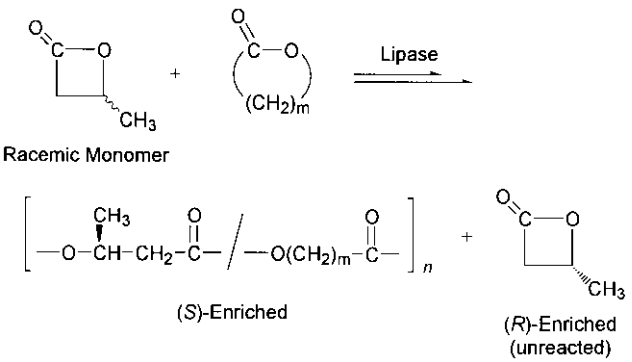
Fig. 11. Expanded ^{13}C NMR spectrum of copolymer obtained by the polymerization of PDL in the presence of poly(ε-CL) using lipase PF catalyst.



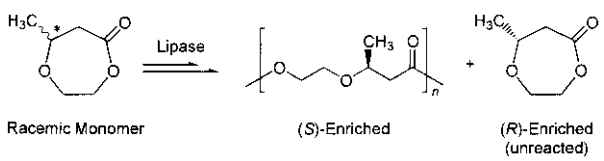
Scheme 10.

Lipase induced enantioselective ring-opening polymerization of racemic lactones. Polyesters with high optical purity were synthesized by lipase CA-catalyzed copolymerization of racemic substituted lactones with achiral unsubstituted lactones. In the lipase CA-catalyzed copolymerization of β-butyrolactone (β-BL) with DDL, (*S*)-β-BL was preferentially reacted to give the (*S*)-enriched optically active copolymer with enantiomeric excess (ee) of β-BL unit = 69% (Scheme 11).[77] δ-Caprolactone (6-membered) was also enantioselectively copolymerized with achiral lactones by the lipase catalyst to give the (*R*)-enriched optically active polyesters whose ee value reached 76%.

Lipase PC induced the enantioselective polymerization of 7-methyl-1,4-dioxepan-5-one (Scheme 12).[52] The initial reaction rate of the *S*-isomer was seven times larger than that of the *R*-isomer, indicating that the enantioselective polymerization took place through lipase catalysis. The *S*-isomer was also polymerized by lipase PF, whereas no polymerization of the *R*-isomer took place with lipase PF. α-Methyl-β-propiolactone was enantioselectively polymerized by lipase PC to produce an optically active (*S*)-enriched polymer with enantiomeric excess ee up to 50%.[78] Recently, lipase CA-catalyzed enantioselective polymerization of fluorinated lactones in the ring-size from 10 to 14 was reported. However, the corresponding hydroxy acid gave an optically inactive polyester.[79]



Scheme 11.



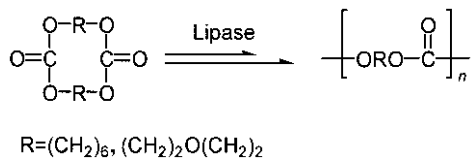
Scheme 12.

We reported that α-substituted lactones, α-methyl-δ-valerolactone and α-methyl-ε-caprolactone, were polymerized by lipase CA catalyst under mild reaction conditions;[80] however, no enantioselection took place. Lipase catalysis induced the ring-opening polymerization of cyclic diesters. The polymerization of ethylene dodecanedioate and ethylene tridecanedioate proceeded through lipase catalysis; lipases CA, PC and PF were highly active for the polymerization.[81] The enzyme origin affected the polymerization behaviors; in using lipase PC catalyst, these bislactones were polymerized faster than ε-CL and DDL, whereas the reactivity of these cyclic diesters was in the middle of the values for ε-CL and DDL in using lipase CA.

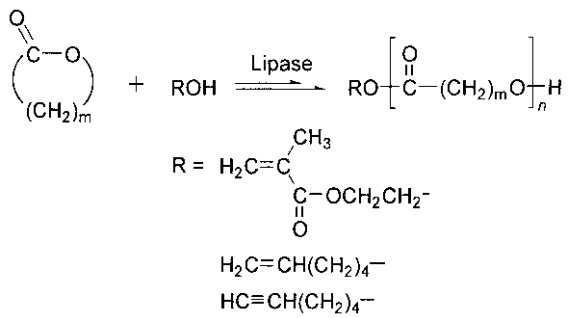
Besides cyclic esters, cyclic carbonates, cyclic dicarbonates, 6-membered depsipeptides, and 5-membered cyclic phosphate were polymerized by lipase catalyst. Lipases CA, PC, PF and PPL induced the ring-opening polymerization of a 6-membered cyclic carbonate, 1,3-dioxan-2-one.[82–84] We found that lipase CA catalyzed the polymerization of cyclic dicarbonates, *cyclo*-[bis(hexamethylene carbonate)] and *cyclo*-[bis(diethylene glycol carbonate)], yielding the corresponding polycarbonates (Scheme 13).[85] The enzymatic copolymerization of the cyclic dicarbonates with lactones proceeded to produce ester–carbonate copolymers.

2-2. Single-Step Synthesis of End-Functionalized Polyesters. Structural control of polymer terminal has been extensively studied since terminal-functionalized polymers, typically macromonomers and telechelics, are often used as prepolymers for synthesis of functional polymers.[86] Various methodologies for synthesis of these polymers have been developed; however, most of them required elaborate and time-consuming procedures. On the other hand, lipase catalysis has provided us with novel methodologies for single-step synthesis of end-functionalized polyesters by facile procedures.

As shown in Scheme 9, an alcohol can act as an initiating species in the ring-opening polymerization of lactones. The lipase CA-catalyzed polymerization of lactones in the presence of functional alcohols produced the end-functionalized polyesters ("initiator method"). In the polymerization of DDL em-



Scheme 13.



Scheme 14.

ploying 2-hydroxyethyl methacrylate as initiator under appropriate reaction conditions, the methacryloyl group was quantitatively introduced at the polymer terminal, yielding the methacryl-type polyester macromonomer (Scheme 14).[87] This methodology was expanded to syntheses of ω-alkenyl- and alkynyl-type macromonomers by using 5-hexen-1-ol and 5-hexyn-1-ol as initiator. This method provided a new methodology of single-step synthesis of polyester macromonomers.

Alkyl glucopyranosides[88,89] and a first generation dendrimer having 6 primary alcohols[90] initiated the polymerization of ε-CL in the presence of lipase CA, where the regioselective initiation (monoacylation of the initiator) took place. In the case of the former, the primary hydroxy group was regioselectively acylated to give the polymer bearing the sugar moiety at the polymer terminal.

We developed a single-step, convenient production of end-functionalized polyesters by lipase-catalyzed polymerization of DDL in the presence of vinyl esters.[91,92] The vinyl ester acted as terminator during the polymerization ("terminator method"). In the use of vinyl methacrylate (0.125 or 0.15 molar amount based on DDL) and lipase PF as terminator and catalyst, respectively, the methacryl group was quantitatively introduced at the polymer terminal to give the methacryl-type polyester macromonomer (Scheme 15). The polymerization in the presence of vinyl 10-undecenoate produced the ω-alkenyl-type macromonomer. The macromonomer production may be explained as follows. During the polymerization of DDL, the enzyme is reacted with the vinyl ester to give the acyl-lipase intermediate, which is subjected to the reaction with the terminal hydroxy group of the lactone polymer to give the macromonomer. Furthermore, the present system can be applied to the synthesis of the telechelics having a carboxylic acid group at both ends by the addition of divinyl sebacate in the reaction mixture.

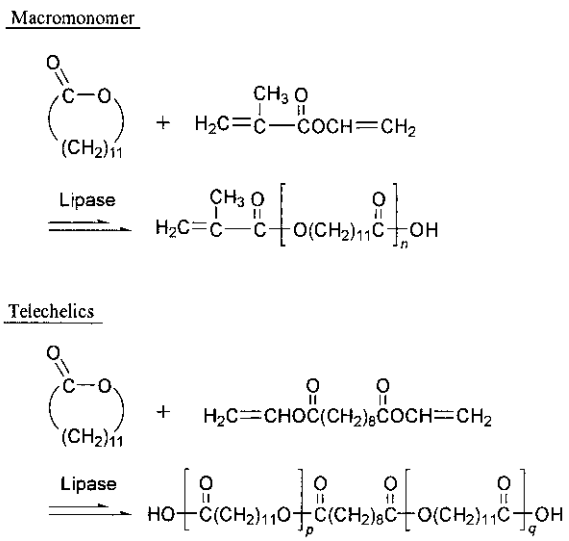
2-3. Polycondensation of Dicarboxylic Acid Derivatives and Glycols. Enzymatic synthesis has been achieved via various combinations of dicarboxylic acid derivatives and glycols. As to the diacid monomer, dicarboxylic acids, activated and non-activated esters, cyclic acid anhydrides, and polyanhydrides were enzymatically reacted with glycols under mild reaction conditions.

When lipase CA was used as catalyst, the polymerization of dicarboxylic acids and α,ω-glycols proceeded without organic solvents, despite the heterogeneous mixture of the monomers and catalyst (lipase and diacid: solid, glycol: solid or liquid depending on the chain length).[93,94] The methylene chain length of 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.[95] A small amount of adjuvant was effective for the polymer production when both monomers were solid at the reaction temperature. A scale-up experiment produced the polyester from adipic acid and 1,6-hexanediol in more than 200 kg yield.[96] This solvent-free system was an environmentally friendly synthetic process of polymeric materials, owing to the mild reaction conditions and no use of organic solvents and toxic catalysts.

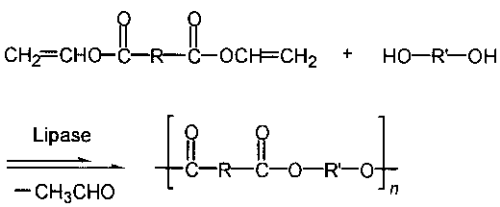
A dehydration reaction is generally realized in non-aqueous media. Since a product water of the dehydration is in equilibrium with starting materials, the solvent water disfavors the dehydration to proceed in an aqueous medium due to "the law of mass action". Nevertheless, we have found that lipase catalysis provided a dehydration polymerization of a dicarboxylic acid and glycol in water.[97] The view of dehydration in an aqueous medium is a new aspect in organic chemistry. In the polymerization of sebacic acid and 1,8-octanediol in distilled water at 45 °C, lipases CA, CC, PC and PF showed high catalytic activity. Both monomers were recovered unchanged in the absence of enzyme (control experiment). These results indicate that the present dehydration polymerization proceeded through enzyme catalysis in the aqueous medium. The chain length of each monomer strongly affected the polymerization behavior; the combination of the monomers with appropriate hydrophobicity was favored for the polymer formation.[98]

Transesterifications using lipase catalyst are often very slow, owing to the reversible nature of the reactions. An irreversible procedure for the lipase-catalyzed acylation using vinyl esters as acylating agent has been developed, where a leaving group of vinyl alcohol tautomerizes to acetaldehyde. In these cases, the reaction with the vinyl ester proceeds much faster to produce the desired compound in higher yields, in comparison with the alkyl esters.

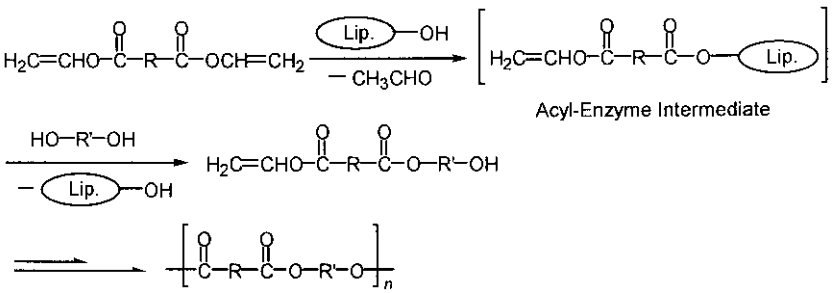
We have first demonstrated the high enzymatic reactivity of divinyl esters for synthesis of polyesters. The polymerization of divinyl adipate and 1,4-butanediol proceeded in the presence of lipase PF at 45 °C (Scheme 16).[99] Under similar reaction conditions, adipic acid and diethyl adipate did not afford



Scheme 15.



Scheme 16.



Scheme 17.

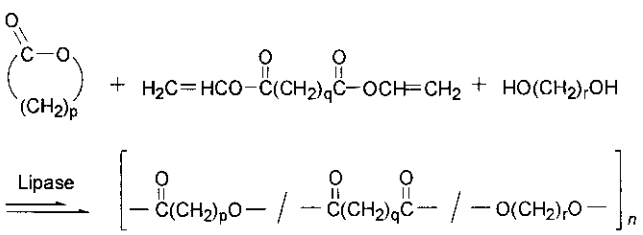
the polymeric materials, indicating the high polymerizability of vinyl esters toward lipase catalyst. Vinyl ester of 12-hydroxydodecanoic acid was also enzymatically polymerized to give the corresponding polyester.[100]

Lipase-catalyzed polymerization of divinyl ester and glycol is proposed to proceed as follows (Scheme 17). First, the hydroxy group of the serine residue nucleophilically attacks the acyl-carbon of the divinyl ester monomer to produce an acyl-enzyme intermediate involving elimination of acetaldehyde. The reaction of the intermediate with the glycol produces 1:1 adduct of both monomers. In the propagation stage, the nucleophilic attack of the terminal hydroxy group takes place on the acyl-enzyme intermediate formed from the vinyl ester group of the monomer, 1:1 adduct, and the subsequent propagation steps keep going similarly.

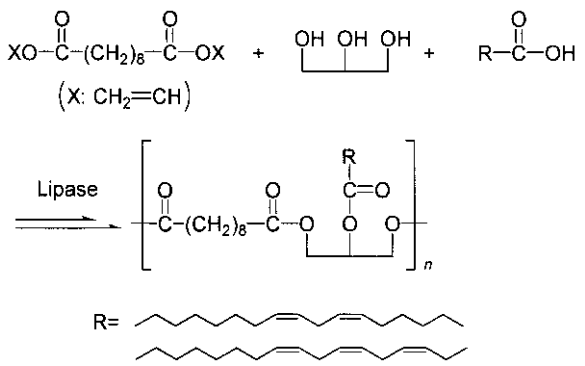
Lipases derived from *Mucor miehei* (lipase MM), CA, PC, and PF showed high catalytic activity toward the polymerization of divinyl adipate or divinyl sebacate with α,ω-glycols with different chain lengths.[101] A combination of divinyl adipate, 1,4-butanediol, and lipase PC afforded the polymer with molecular weight of 2.1×10^4. The yield of the polymer from divinyl sebacate was higher than that from divinyl adipate, whereas the opposite tendency was observed in the polymer molecular weight.

Aromatic polyesters were enzymatically synthesized by the polycondensation of divinyl esters of isophthalic acid, terephthalic acid, and 1,4-benzenediacetic acid with glycols by using lipase CA catalyst.[102] The highest molecular weight (7.2×10^3) was attained from a combination of divinyl isophthalate and 1,10-decanediol. Enzymatic polymerization of divinyl sebacate and 1,4-benzenedimethanol also afforded the aromatic polyester.

Lipase-catalyzed copolymerization of lactones, divinyl esters, and glycols produced ester copolymers with molecular weights higher than 1×10^4 (Scheme 18).[103] Lipases CA and PC showed high catalytic activity for the present copolymerization. From ^{13}C-NMR analysis, the resulting product was not a mixture of homopolymers, but a copolymer derived from the monomers, indicating that two different modes of polymerization, ring-opening polymerization and polycondensation, simultaneously take place through enzyme catalysis in one-pot to produce ester copolymers.



Scheme 18.



Scheme 19.

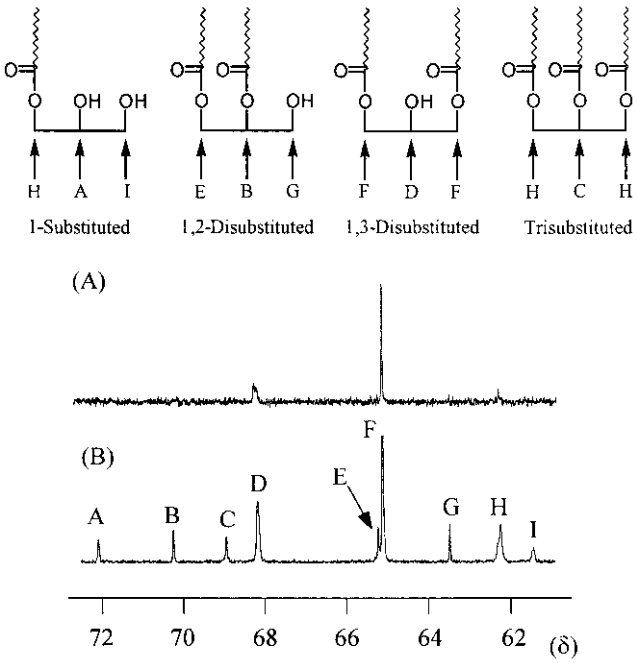
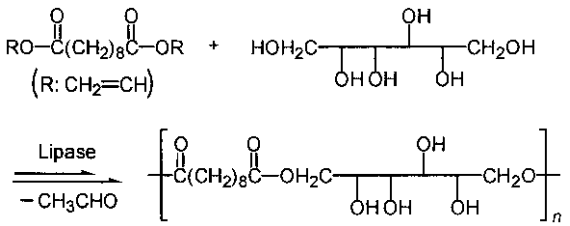


Fig. 12. Expanded ^{13}C NMR spectra of polymer from divinyl sebacate and glycerol using lipase CA catalyst at (A) 45 °C and (B) 60 °C.



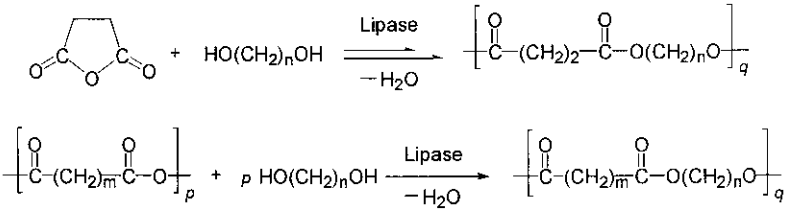
Scheme 20.

Polymerization of divinyl esters and triols regioselectively proceeded via lipase catalysis to give soluble polymers with relatively high molecular weights.[104,105] NMR analysis of the polymer obtained from divinyl sebacate and glycerol using lipase CA catalyst at 60 °C showed that 1,3-diglyceride was a main unit and that a small amount of the branching unit (triglyceride) was contained (Fig. 12). The regioselectivity of the acylation between primary and secondary hydroxy groups was 74:26. In the polymerization at 45 °C, the regioselectivity was perfectly controlled to give a linear polymer consisting exclusively of 1,3-glyceride units.

New crosslinkable polyesters were synthesized by lipase-catalyzed polymerization of divinyl sebacate and glycerol in the presence of unsaturated higher fatty acids derived from renewable plant oils (Scheme 19).[106] Single-step synthesis of the crosslinkable polyester having an unsaturated group in the side chain was achieved by using lipase CA as catalyst. The polymerization under reduced pressure improved the polymer yield and molecular weight. The curing of the polymer obtained using linoleic or linolenic acid proceeded by cobalt naphthenate catalyst or thermal treatment to give a crosslinked transparent film.

A sugar-containing polyester was synthesized by the polymerization of divinyl sebacate and D-glucitol using lipase CA as catalyst in acetonitrile (Scheme 20).[107] NMR analysis showed that D-glucitol was regioselectively acylated at 1- and 6-positions. Mannitol and *meso*-erythritol were also regioselectively polymerized with divinyl sebacate. Another sugar-containing polyester was obtained by protease-catalyzed polycondensation of sucrose with bis(2,2,2-trifluoroethyl) adipate.[108] The polymer possessed ester linkages at 6- and 1′-positions on the sucrose.

Anhydride derivatives are also good acylating reagents through lipase catalysis. A new type of enzymatic polymerization involving lipase-catalyzed ring-opening poly(addition-condensation) of cyclic acid anhydride with glycols was demonstrated (Scheme 21).[109] The polymerization of succinic anhydride with 1,8-octanediol using lipase PF catalyst proceeded at room temperature to produce the polyester. Enzymatic synthesis of polyesters was also achieved by the reaction of polyanhydrides and glycols. The reaction of poly(azelaic anhydride) and 1,8-octanediol took place by using lipase CA catalyst to give the corresponding polyester with molecular weight of several thousands.[110] The highly branched polyester



Scheme 21.

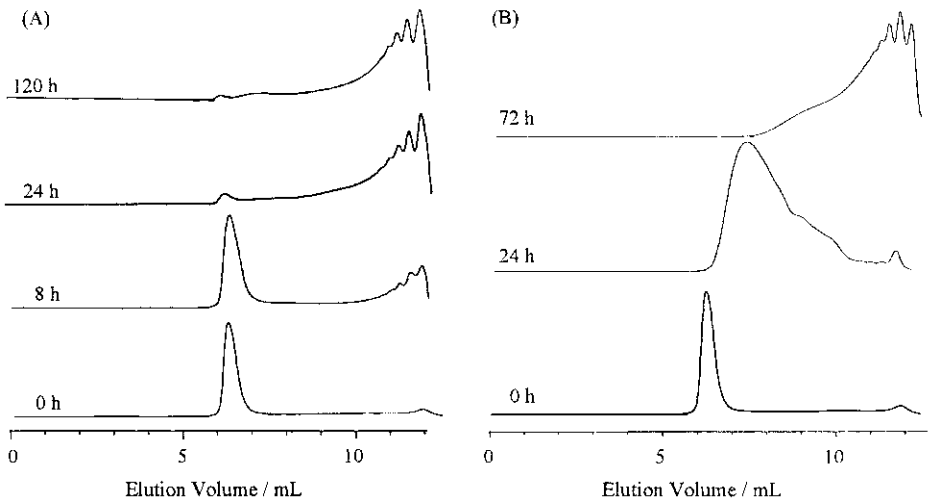
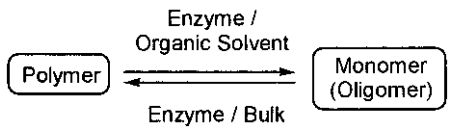


Fig. 13. Size exclusion chromatography traces of degradation products from poly(ε-CL) (mol wt = 4×10^4) using (A) lipase CA catalyst and (B) *p*-toluenesulfonic acid (0.05 molar amount for monomer unit of poly(ε-CL)). The degradation was performed in toluene at 60 °C in toluene.

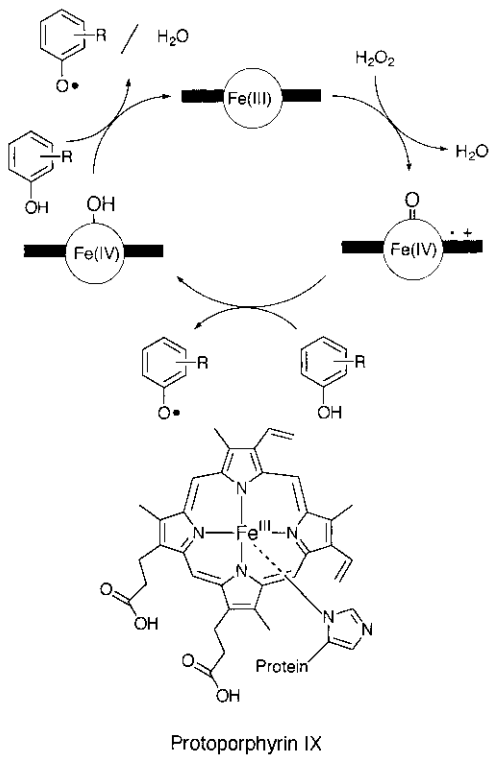
with branching factor > 0.7 was synthesized by the lipase CA-catalyzed reaction of poly(azelaic anhydride) and triols such as glycerol.

2-4. Chemical Recycling of Polyesters by Lipase Catalyst. Worldwide potential demands for recycling of polymeric materials are quite significant. Among recycling methods, chemical recycling is the most desirable because starting materials can be reproduced. However, industrial examples of chemical recycling are limited (alcoholysis of PET and PBT) and their processes normally consume much energy. Biodegradable polymers are expected as an alternative to traditional non-biodegradable polymers. These polymers are subjected to degradation by living organisms, whereas the degradation products are not directly converted to the original polymers. We first proposed a new concept of chemical recycling of polymers using lipase catalyst.

Aliphatic polyesters were subjected to hydrolytic degradation by lipase catalyst in organic solvents. The lipase CA-catalyzed degradation of poly(ε-CL) with molecular weight = 4×10^4 readily took place in toluene at 60 °C to give oligomers with molecular weight less than 500 (Fig. 13). The degradation behavior catalyzed by lipase was quite different than an acid-catalyzed degradation (random bond cleavage of polymer). After the removal of the solvent from the reaction mixture, the residual oligomer was polymerized in the presence of the same catalyst of lipase. These data provide a basic concept that the degradation–polymerization could be controlled by presence or absence of the solvent, providing a new methodology of plastics recycling (Scheme 22).



Scheme 22.



Scheme 23.

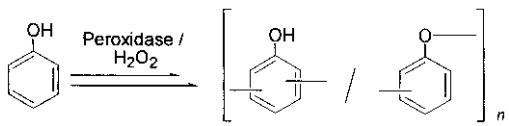
3. Polyaromatics

In living cells, various oxidoreductases play an important role in maintaining the metabolism of living systems. So far, several oxidoreductases, peroxidase, laccase, bilirubin oxidase etc. have been reported to catalyze oxidation polymerization of phenol and aniline derivatives; among them, peroxidase is most often used. Peroxidase is an enzyme whose catalysis is an oxidation of a donor to an oxidized donor by the action of hydrogen peroxide, liberating two water molecules for a catalytic cycle. Horseradish peroxidase (HRP) is a single-chain β-type hemoprotein that catalyzes the decomposition of hydrogen peroxide at the expense of aromatic proton donors. Catalytic cycle of HRP for a phenol substrate is shown in Scheme 23.[9]

3-1. Enzymatic Polymerization of Phenols. Phenol–formaldehyde resins using prepolymers such as novolaks and resols are widely used in industrial fields. These resins show excellent toughness and temperature-resistant properties. However, the toxic nature of formaldehyde creates problems in their manufacture and use. Therefore, an alternative process for preparation of phenol polymers without using formaldehyde is strongly desired.

In 1987, enzymatic syntheses of a new class of polyphenols have been first reported.[114] An oxidative polymerization of *p*-phenylphenol using HRP as catalyst was carried out in a mixture of water and water-miscible solvents such as 1,4-dioxane, acetone, *N*,*N*-dimethylformamide (DMF), and methyl formate to give powdery polymeric materials. The reaction medium composition greatly affected the molecular weight, and the highest molecular weight (2.6×10^4) was achieved in 85% 1,4-dioxane.

In the case of phenol, the simplest and most important phenolic compound in industrial fields, conventional polymerization catalysts afford an insoluble product with non-controlled structure since phenol is a multifunctional monomer for oxidative polymerization.[115] On the other hand, we have found that the peroxidase catalysis induced the polymerization in an aqueous organic solvent to give a powdery polymer consisting of phenylene and oxyphenylene units showing relatively high thermal stability (Scheme 24)[116–121]. HRP and soybean peroxidase (SBP) were active as catalyst for the polymerization in the aqueous 1,4-dioxane;[116–118] however, the resulting polymer showed low solubility; the polymer was partly soluble in DMF and dimethyl sulfoxide, insoluble in other common organic solvents. The solubility was much improved by using a mixed solvent of buffer and methanol, producing the DMF-soluble polymer with controlled molecular weight of 2100–6000 in



Scheme 24.

good yields. Furthermore, the unit ratio (regioselectivity) could be controlled by changing the solvent composition; a polymer containing the phenylene units from 32 to 66% was obtained.[119,120]

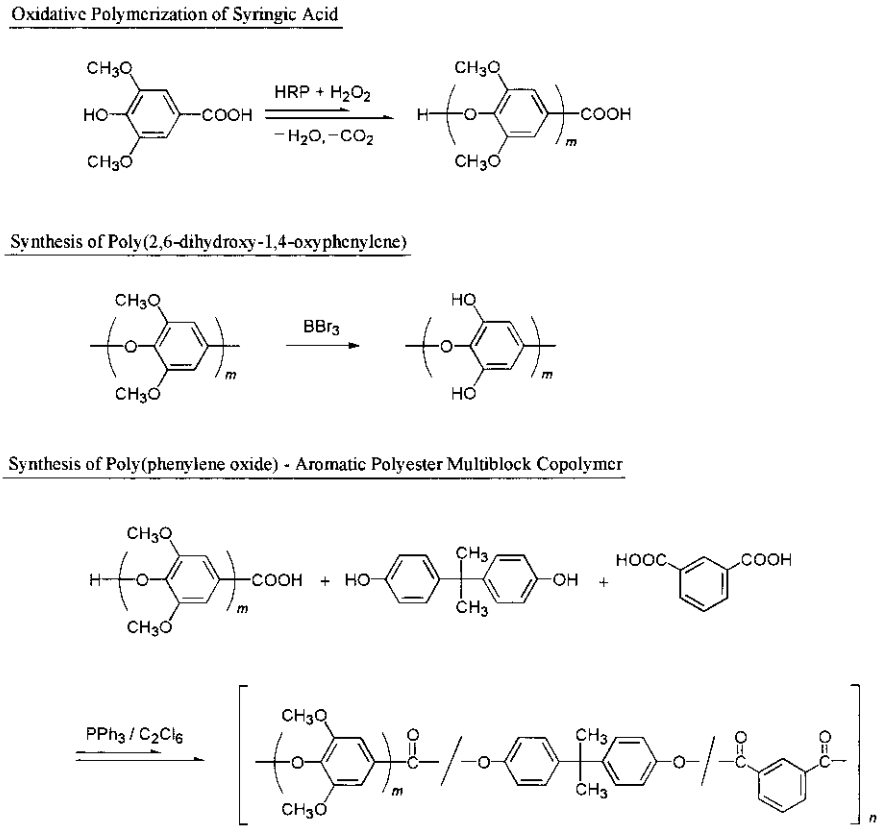
So far, various alkylphenol derivatives have been polymerized through peroxidase catalysis in the aqueous organic solvent. For the case of a combination of *p*-*n*-alkylphenols and HRP, the polymer yield increased as the chain length of the alkyl group increased from 1 to 5[122,123]. All cresol isomers were polymerized by HRP catalyst,[124] which also produced poly(*p*-isopropylphenol) efficiently, whereas *o*- and *m*-isopropylphenols were not polymerized under similar reaction conditions. Poly(*p*-*n*-alkylphenol)s prepared in the aqueous 1,4-dioxane showed low solubility toward common organic solvents, and the molecular weight was in the range of several thousands.

As to *m*-alkyl substituted phenols, the soluble polyphenols were obtained by HRP or SBP catalyst in the aqueous methanol.[125] Enzymatically synthesized poly(*m*-cresol) had a glass transition temperature (T_g) higher than 200 °C. The enzyme origin strongly influenced the polymer yield; HRP readily polymerized the monomer having a small substituent, whereas in the case of large substituent monomers, the higher yield was achieved by using SBP as catalyst.

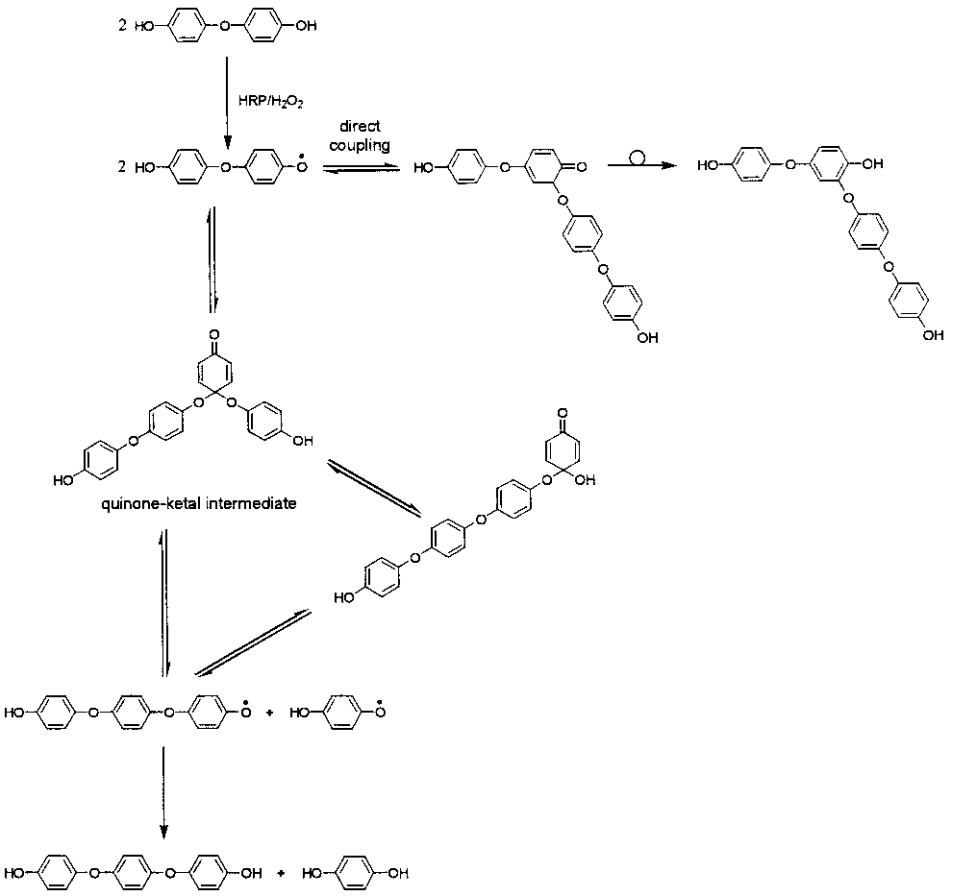
Advantages for enzymatic synthesis of polyphenols are summarized as follows: (i) the polymerization of phenols proceeds under mild reaction conditions without use of toxic reagents (environmentally benign process); (ii) phenol monomers having various substituents are polymerized to give a new class of functional polyaromatics; (iii) the structure and solubility of the polymer can be controlled by changing the reaction conditions; (iv) the procedures of the polymerization as well as the polymer isolation are very convenient.

3-2. Enzymatic Synthesis of New Functional Polyphenols. Poly(oxy-2,6-dimethyl-1,4-phenylene) (poly(phenylene oxide), PPO) is widely used as a high-performance engineering plastic, since the polymer has excellent chemical and physical properties, e.g., a high glass transition temperature (ca. 210 °C) and mechanical toughness. PPO was first prepared from 2,6-dimethylphenol monomer using a copper/amine catalyst system.[115,126] 2,6-Dimethylphenol was also polymerized through HRP catalysis to give the polymer consisting of exclusively oxy-1,4-phenylene unit;[127] on the other hand, small amounts of Mannich-base and 3,5,3′5′-tetramethyl-4,4′-diphenoquinone units are contained in the commercially available PPO. The polymerization also proceeded in the presence of laccase derived from *Pycnoporus coccineus* under air without the addition of hydrogen peroxide.

Syringaldehyde is abundantly present in plants as their glycosidic derivatives. We have achieved PPO synthesis by the enzymatic polymerization of syringic acid, an acidic form of syringaldehyde (Scheme 25).[100,121,128–130] In the polymerization using HRP catalyst in a mixture of acetone/acetate buffer (pH 5) (40:60 vol%), polymeric materials with a molecular weight of 1.3×10^4 were obtained in 79% yield. From NMR, IR, and MALDI-TOF mass analyses, the polymer was found to consist of exclusively 1,4-oxyphenylene unit and to have a phenolic hydroxy group at one terminal end and a benzoic acid



Scheme 25.



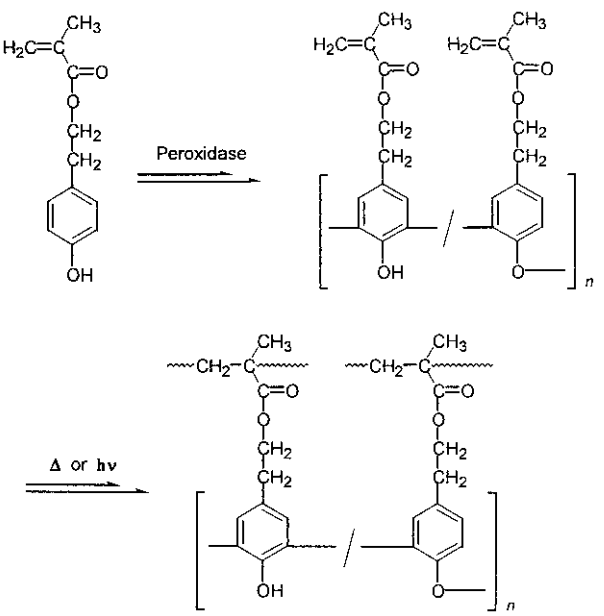
Scheme 26.

group at the other, indicating that the polymerization involved elimination of carbon dioxide and hydrogen from the monomer. SBP and laccase were also active for the polymerization. 4-Hydroxy-3,5-dimethylbenzoic acid was also enzymatically polymerized to give PPO, on the other hand, the polymerization of non-substituted 4-hydroxybenzoic acid did not occur under the similar reaction conditions.

As one possible application, the polyether from syringic acid was converted to a new PPO derivative, poly(oxy-2,6-dihydroxy-1,4-phenylene), by demethylation with an excess of boron tribromide in dichloromethane.[131] NMR and IR analyses showed that the extent of the demethylation was 93% and the polymer was composed of oxy-2,6-dihydroxy-1,4-phenylene unit. The polymer was stable below 300 °C under nitrogen. The resulting polymer is useful as a starting material for syntheses of functional polymers since it has a reactive phenolic group and a PPO backbone having high thermal stability and chemical resistance.

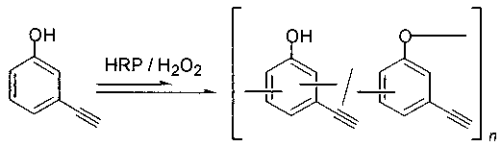
By utilizing two different terminal functional groups of the polymer, one can synthesize a new functional polymeric material containing PPO unit. Polycondensation of bisphenol-A, isophthalic acid, and the polymer in the presence of triphenylphosphine/hexachloroethane (coupling agent) afforded PPO-aromatic polyester multiblock copolymers[132]. From TG analysis, the multiblock copolymer was found to show relatively high thermal stability.

We have first demonstrated that, in the HRP-catalyzed oxi-



Scheme 27.

dative polymerization of 4,4′-oxybisphenol in aqueous methanol, α-hydroxy-ω-hydroxyoligo(oxy-1,4-phenylene)s were obtained in moderate yields[133]. During the reaction, hydroquinone was formed. Scheme 26 shows the postulated



Scheme 28.

mechanism of the trimer formation; the redistribution and/or rearrangement of the quinone-ketal intermediate take place, involving the elimination of hydroquinone.

HRP catalysis induced a chemoselective polymerization of a phenol derivative having a methacryloyl group. Only the phenol moiety was polymerized, without involving vinyl polymerization of methacryloyl, to give a polymer having the methacryloyl group in the side chain (Scheme 27). The resulting polymer was readily subjected to thermal and photochemical curings.

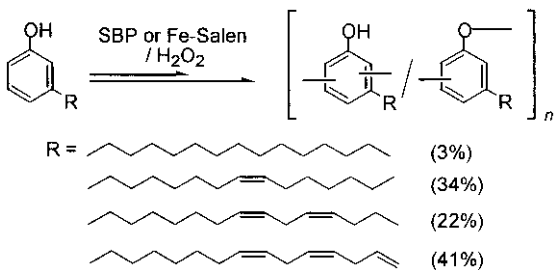
A phenol with an acetylenic substituent in the meta position was also chemoselectively polymerized to give the polyphenol having the acetylenic group (Scheme 28)[135]. The resulting polymer was converted to carbonized polymer in a much higher yield than enzymatically synthesized poly(*m*-cresol) and is expected to have potential applications as a reactive starting polymer.

Cardanol, a main component obtained by thermal treatment of cashew nut shell liquid (CNSL), is a phenol derivative having the meta substituent of a C15 unsaturated hydrocarbon chain with one to three double bonds as the major. Since CNSL is nearly one-third of the total nut weight, much amount of CNSL is obtained as by-products from mechanical processes for the edible use of the cashew kernel. Only a small part of cardanol obtained in the production of cashew kernel is used in industrial fields, though it has various potential industrial utilizations such as resins, friction-lining materials, and surface coatings. Therefore, development of new applications for cardanol is very attractive.

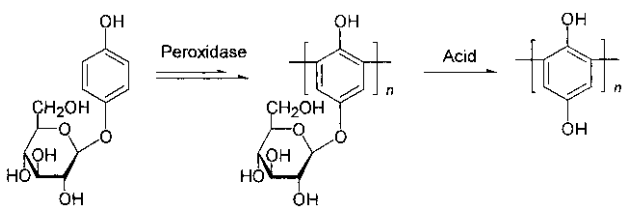
We synthesized a new crosslinkable polymer by the SBP-catalyzed polymerization of cardanol (Scheme 29).[100,121,130,136] The polymerization in a mixture of acetone/buffer of pH 7 (75:25%) produced oily products and the yield and molecular weight of the methanol-insoluble part were 21% and 4800, respectively. The polymer was soluble in common polar organic solvents and possessed the unsaturated group in the side chain. FT-IR analysis showed that the carbon-carbon unsaturated group in the side chain of cardanol did not change during the polymerization.

A thermally-curable polyphenol was synthesized by peroxidase-catalyzed polymerization of bisphenol-A.[137] The polymer was crosslinked at 150–200 °C and the curing improved the thermal stability of the polymer. The reaction with epoxy resin produced the insoluble network polymer.

A natural phenol, 4-hydroxyphenyl β-D-glucopyranoside (arbutin), was oxidatively polymerized using peroxidase catalyst in a buffer solution, yielding water-soluble polymers with molecular weight ranging from 1600 to 3200 (Scheme 30).[138]



Scheme 29.



Scheme 30.

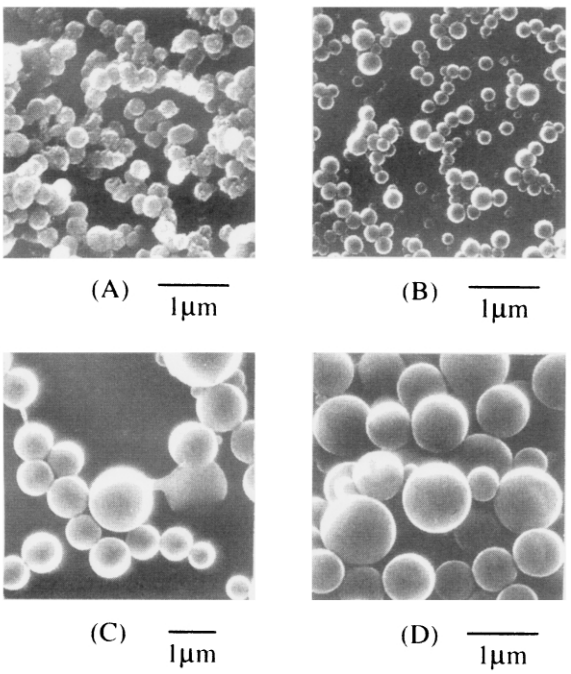
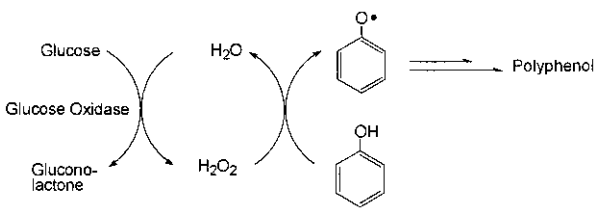


Fig. 14. SEM photographs of polymer particles from (A) phenol, (B) *m*-cresol, (C) *p*-cresol, and (D) *p*-phenylphenol. Particles were obtained by the dispersion polymerization of phenol derivative (5 mmol) using HRP catalyst (10 mg) in the presence of PVME (25 wt% for the monomer) in a mixture of 1,4-dioxane and phosphate buffer (pH 7) (25mL, 60:40 vol%) at room temperature for 24 h.



Scheme 31.

Acidic deglycosylation of the resulting polymer afforded soluble poly(hydroquinone), which may be of *ortho–ortho* coupling structure. We reported chemoenzymatic synthesis of poly(hydroquinone) from 4-hydroxyphenyl benzoate, whose structure was different with that from arbutin.[139]

Fluorinated phenols, 3- and 4-fluorophenols, and 2,6-difluorophenol, were subjected to peroxidase-catalyzed polymerization, yielding fluorine-containing polymerizations. During the polymerization, elimination of some fluorine atoms took place to give the polymers with complicated structures.[140]

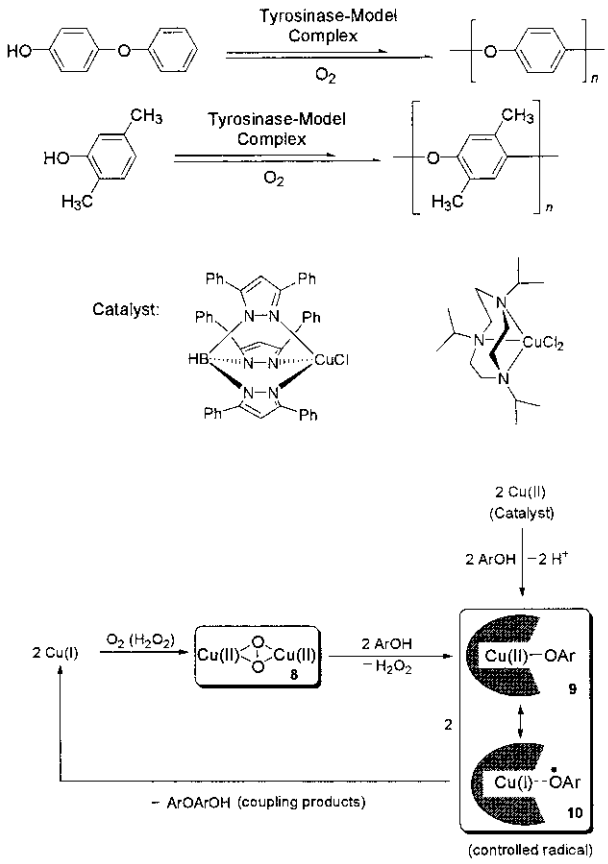
Morphology of the enzymatically synthesized polyphenol was controlled under the selected reaction conditions. Monodisperse polyphenol particles in the sub-micron range were produced by HRP-catalyzed dispersion polymerization of phenol in 1,4-dioxane-phosphate buffer (3:2 vol/vol) using poly(vinyl methyl ether) (PVME) as stabilizer (Fig. 14).[141–143] The particle size could be controlled by the stabilizer concentration and solvent composition. Thermal treatment of these particles afforded uniform carbon particles. The particles could be obtained from various phenol monomers such as *m*-cresol and *p*-phenylphenol.

A bienzymatic system (glucose oxidase+HRP) was used as catalyst for the polyphenol synthesis (Scheme 31).[144] This system induced the polymerization of phenol in the presence of glucose without the addition of hydrogen peroxide to produce the polymer in a moderate yield. Hydrogen peroxide was formed *in situ* by the oxidation of glucose catalyzed by glucose oxidase, which acted as oxidizing agent for the polymerization.

3-3. Polymerization Using Enzyme-Model Complexes as Catalyst. Metal complexes containing a catalytic site of enzymes or its modified moiety have been used as catalyst for oxidative polymerizations. Regioselective oxidative polymerization of phenol leading to *2,6-unsubstituted* PPO has not been achieved. This is due to the low selectivity in "electrophilic" or "free" phenoxyl radical coupling. We have first achieved the regioselective polymerization of 4-phenoxyphenol using tyrosinase model complexes as catalyst to give unsubstituted crystalline PPO having melting points (Scheme 32).[121,145–149] The high selectivity is explained as follows. "Nucleophillic" μ-η^2:η^2-peroxo dicopper(II) complex **8** is generated as the sole active oxygen complex from the catalyst, and abstracts a proton (not a hydrogen atom) from the monomer to give phenoxo–copper(II) complex **9**, equivalent to phenoxyl radical–copper(I) complex **10**. Intermediates **9** and/or **10** are not "free" radicals, but "controlled" radicals, and hence, regioselectivity of the subsequent coupling is regulated.

The same catalyst system induced an oxidative polymerization of 2,5-dimethylphenol, giving rise to poly(oxy-2,5-dimethyl-1,4-phenylene) having a melting point of 275–308 °C.[121,150] From *o*- and *m*-cresols, polymers consisting mainly of oxy-1,4-phenylene unit were also formed.[151,152]

We regarded iron-*N*,*N*′-ethylenebis(salicylideneamine) (Fe-salen) as a model complex of peroxidase and used it as catalyst for oxidative polymerization of phenols. Fe-salen catalyzed an oxidative polymerization of *p*-*t*-butylphenol and bisphenol-A, yielding soluble polyphenols.[153] 2,6-Dimethyl- and 2,6-difluorophenols were polymerized by Fe-salen catalyst to give PPO derivatives.[154,155] The latter polymer showed crystallinity, with



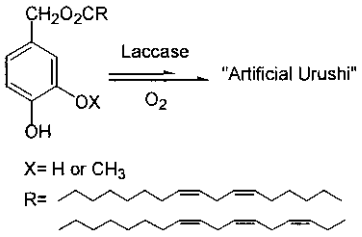
Scheme 32.

melting point higher than 250 °C.

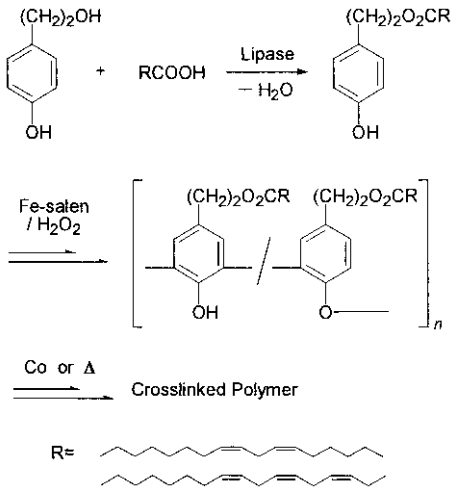
We polymerized cardanol using Fe-salen as catalyst to give a soluble polyphenol containing the unsaturated alkyl group in the side chain.[121,156] The polymer was subjected to hardening by cobalt naphthenate catalyst or thermal treatment, yielding a crosslinked film with a high gloss surface. The hardness of the cured film, evaluated by a dynamic microhardness tester, reached nearly 100 N mm^{-2} after 7 days; such value is comparable to that of commercial cardanol-formaldehyde coating materials.

3-4. Preparation of Artificial Urushi. Urushi is a typical Japanese traditional coating showing excellent toughness and brilliance for a long period. In the early days of this century, pioneering works by Majima revealed that the main important components of urushi are "urushiols", whose structure is a catechol derivative directly linked to unsaturated hydrocarbon chains consisting of a mixture of monoenes, dienes, and trienes at 3- or 4-position of catechol.[157–159] Film-forming of the urushiol proceeds under air at room temperature without organic solvents; hence, urushi seems very desirable for coating materials from the environmental standpoint. However, few modeling studies of urushi have been attempted. This is mainly due to the difficulty in preparation of the urushiol.

We have created a novel system of enzymatic polymerization, i.e., a laccase-catalyzed crosslinking reaction of new "urushiol analogues" for the preparation of "artificial urushi" (Scheme 33).[160,161] Single-step synthesis of the urushiol analogues was achieved by using lipase as catalyst. These com-



Scheme 33.



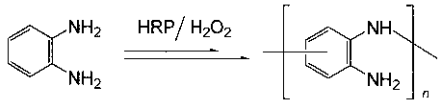
Scheme 34.

pounds were cured in the presence of laccase catalyst under mild reaction conditions without use of organic solvents to produce the crosslinked polymeric film with high gloss surface and good elastic properties. Such multienzymatic processes are highly significant as a fundamental study for an alternative of conventional commercial coatings utilizing many organic solvents and severe hardening conditions.

Furthermore, we designed new crosslinkable polyphenols on the basis of model "urushi" and synthesized them by the Fe-salen-catalyzed oxidative polymerization of other urushiol analogues (Scheme 34).[162] Such polyphenols were readily cured to give crosslinked polymeric films with high gloss surface.

3-5. Enzymatic Synthesis of Polyaniline Derivatives. Peroxidase also catalyzed oxidative polymerization of aniline derivatives. HRP-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 (Scheme 35).[163] 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*-pheneylenediamine, FT-IR analysis showed the formation of the corresponding copolymer.[164]

In the HRP-catalyzed polymerization of aniline under neutral conditions, the polymer with a complicated structure was obtained in low yields. In using sulfonated polystyrene (SPS) as template, on the other hand, the electroactive form of polyaniline was enzymatically formed.[165]



Scheme 35.

Conclusion

Our recent results on *in vitro* polymer syntheses using an isolated enzyme as catalyst via non-biosynthetic pathways ("enzymatic polymerization") have been overviewed. Besides such topics, protease-catalyzed synthesis of oligopeptides from esters of amino acid[166] and laccase-catalyzed polymerization of vinyl monomers[167] are new and promising routes for functional polymers. These enzymatic routes possess several advantages in comparison with previous biological and chemical processes: (i) structural variations of monomers and polymers, (ii) non-toxic catalyst and mild reaction conditions, and (iii) enantio-, regio-, chemo-, and choro-selective polymerizations to produce functional polymers, which are very difficult to obtain by conventional methodologies. In addition, with the advent of genetic engineering, it is becoming possible to produce a wider range of enzymes on a large scale, thus, the number of enzymes available for synthetic reactions is expanding. Enzymatic polymerizations, therefore, are expected to create a new area of precision polymer syntheses. Furthermore, the enzymatic polymerizations have a large potential as an environmentally friendly synthetic process of polymeric materials, which provides with a good example to achieve "green polymer chemistry".[12,14,110]

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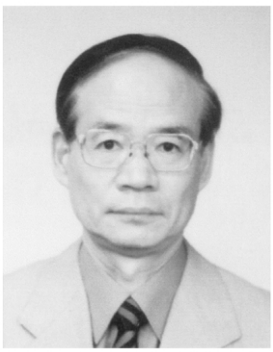

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