Enzymatic Polymerization

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I. Introduction

There has been an exponential increase in interest in the area of in vitro enzyme-catalyzed organic reactions, since many families of enzymes can be utilized for transformation of not only their natural substrates but a wide range of unnatural compounds, yielding a variety of useful materials.[1] Employing enzymes in organic synthesis has several advantages as follows: (i) catalysis under mild reaction conditions with regard to temperature, pressure, and pH, which often lead to remarkable energy efficiency; (ii) high enantio-, regio-, and chemoselectivities as well as regulation of stereochemistry providing development of new reactions to functional compounds for pharmaceuticals and agrichemicals; (iii) nontoxic natural catalyst with "green" appeal in commercial benefit and ecological requirement.

All naturally occurring polymers are produced in vivo by enzymatic catalysis. Recently, in vitro synthesis of polymers through enzymatic catalysis ("enzymatic polymerization") has been extensively developed.[2] Enzyme catalysis has provided a new synthetic strategy for useful polymers, most of which are otherwise very difficult to produce by conventional chemical catalysts. In vitro enzymatic syntheses of polymers via nonbiosynthetic pathways, therefore, are recognized as a new area of precision polymer syntheses.

Furthermore, the enzymatic polymerizations may greatly contribute to global sustainability without depletion of important resources by using nonpetrochemical renewable resources as starting substrates of functional polymeric materials. In the enzymatic polymerizations, the product polymers can be obtained under mild reaction conditions without using toxic reagents. Therefore, the enzymatic polymerization can be regarded as an environmentally friendly synthetic process of polymeric materials, providing a good example to achieve "green polymer chemistry".[3]

The present article overviews recent advances in enzymatic polymerizations. We define enzymatic polymerization as chemical polymer synthesis in vitro (in test tubes) via nonbiosynthetic (nonmetabolic) pathways catalyzed by an isolated enzyme. Accordingly, polymer syntheses by employing a living system like fermentation and *E. coli* using processes are not included.

More than 100 years ago (1894), Emil Fischer proposed a "Key and Lock" theory as to the specific substrate selectivity by the enzyme, which is presently understood as molecular recognition of the substrate by the enzyme through supramolecular interactions. If the enzymatic reaction takes place in vivo, it is always involved to recognize the substrate by the enzyme. This is also true for enzymatic reactions in vitro. However, readers will see in this article that the substrate−enzyme relationship is not as strict as the key−lock relationship, but enzymes are dynamic and sometimes very generous in recognizing even unnatural substrates in vitro. This situation allows enzymes to catalyze the synthesis of not

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Hiroshi Uyama was born in Kobe, Japan, in 1962. He received his B.S. (1985) and M.S. (1987) degrees from Kyoto University. In 1988, he joined the Department of Applied Chemistry, Tohoku University, as Research Associate. He obtained Ph.D. degree under the direction of Professor Shiro Kobayashi in 1991. He moved to the Department of Materials Chemistry, Kyoto University, in 1997. In 2000, he was appointed Associate Professor of the same department. He was honored as the recipient of the Award of the Society of Polymer Science, Japan, for the Outstanding Paper published in the *Polymer Journal* in 1995 and the Chemical Society of Japan Award for Young Chemists in 1997.

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only some natural polymers but also a variety of unnatural polymers. Thus, the target macromolecules for the enzymatic polymerization have been polysaccharides, polyesters, polycarbonates, poly(amino acid)s, polyaromatics, vinyl polymers, etc. All the enzymes are generally classified into six groups, and typical polymers produced with catalysis by respective enzymes are given in Table 1. Here, enzymatic polymerizations are described according to the nature of catalyst enzymes.

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, polyesters
hydrolases	polysaccharides, polyesters, polycarbonates, poly(amino acid)s
lyases	
isomerases	
ligases	

II. Oxidoreductases

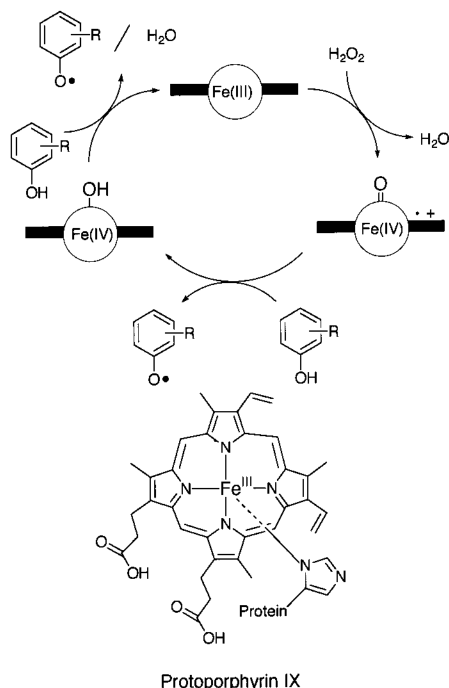
In living cells, various oxidoreductases play an important role in maintaining the metabolism of living systems. Most of oxidoreductases contain low-valent metals as the catalytic center. In vitro enzymatic oxidoreductions have afforded functional organic materials.[1] Recently, some oxidoreductases such as peroxidase, laccase, and bilirubin oxidase have received much attention as catalyst for the oxidative polymerizations of phenol and aniline derivatives to produce novel polyaromatics.

A. Peroxidases

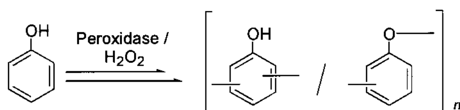
1. Oxidative Polymerization of Phenol Derivatives

Peroxidase catalysis is an oxidation of a donor to an oxidized donor by the action of hydrogen peroxide, liberating two water molecules. Horseradish peroxidase (HRP) is a single-chain β-type hemoprotein that catalyzes the decomposition of hydrogen peroxide at the expense of aromatic proton donors. HRP is a Fe-containing porphyrin-type structure and is well-

Scheme 1



Scheme 2



known to catalyze coupling of a number of phenol and aniline derivatives using hydrogen peroxide as oxidant. The catalytic cycle of HRP for a phenol substrate is shown in Scheme 1. The peroxidase-catalyzed oxidation proceeds fast in aqueous solutions, giving rise to the formation of oligomeric compounds. The resulting oligomers often show low solubility toward the solvent, thereby preventing the further formation of higher molecular weight polymer.

In a mixture of water and water-miscible solvents such as acetone, 1,4-dioxane, and methanol, peroxidase could act as catalyst for oxidative polymerization of various phenol derivatives, yielding a new class of polyaromatics.[4] The polymerization proceeds at room temperature, and during the polymerization, powdery polymers are often precipitated, which are readily collected after the polymerization.

Phenol, the simplest and most important phenolic compound in industrial fields, is a multifunctional monomer for oxidative polymerization, and hence, conventional polymerization catalysts afford an insoluble product with uncontrolled structure. On the other hand, the peroxidase catalysis induced the polymerization in aqueous organic solvent to give a powdery polymer consisting of phenylene and oxyphenylene units showing relatively high thermal stability (Scheme 2).[5,6] In the HRP and soybean peroxidase (SBP)-catalyzed polymerization in the aqueous 1,4-dioxane, the resulting polymer showed low solubility; the polymer was partly soluble in *N,N*-dimethylformamide (DMF) and dimethyl sulfoxide and insoluble in other common organic solvents.[5] On the other hand, the aqueous methanol solvent afforded the DMF-soluble polymer with a number-average molecular weight (M_n) of 2100−6000 in good yields.[6] Furthermore, the unit ratio (regioselectivity) could be controlled by changing the solvent composition to give a polymer with the phenylene unit ranging from 32% to 66%. Molecular weight control of the polyphenol was achieved by the copolymerization with 2,4-dimethylphenol.[7]

The polymerization behaviors and properties of the polyphenols depend on the monomer structure, solvent composition, and enzyme origin. In the HRP-catalyzed polymerization of *p*-*n*-alkylphenols in aqueous 1,4-dioxane, the polymer yield increased as the chain length of the alkyl group increased from 1 to 5.[8] The molecular weight was on the order of several thousands. HRP catalyzed the oxidative polymerization of all cresol isomers,[9] whereas among *o*-, *m*-, and *p*-isopropylphenol isomers, only *p*-isopropylphenol was polymerized by HRP catalysis. Poly(*p*-alkylphenol)s obtained in the aqueous 1,4-dioxane often showed low solubility toward organic solvents; however, soluble oligomers with molecular weight less than 1000 were formed from *p*-ethylphenol in aqueous DMF.[10]

As for *m*-alkyl-substituted phenols, soluble polyphenols were obtained by HRP or SBP catalyst in aqueous methanol.[11] 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.

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.[12] However, the toxic nature of formaldehyde causes problems in their manufacture and practical use. Therefore, the enzymatic processes are highly expected as an alternative for preparation of phenol polymers without using formaldehyde. Advantages for enzymatic synthesis of useful polyphenols are summarized as follows:[13] (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 facile.

Numerical and Monte Carlo simulations of the peroxidase-catalyzed polymerization of phenols were demonstrated.[14] The monomer reactivity, molecular weight, and index were simulated for precise control of the polymerization of bisphenol A. In aqueous 1,4-dioxane, aggregates from *p*-phenylphenol were detected by difference UV absorption spectroscopy.[15] Such aggregate formation might elucidate the specific solvent effects in the enzymatic polymerization of phenols.

The mechanistic study of the HRP-catalyzed oxidative polymerization was performed by using in situ

NMR spectroscopy.[16] In the polymerization of 8-hydroxyquinoline-5-sulfonate, the 2-, 4-, and 7-positions were involved in the oxidative coupling with the order of preference being $7 \geq 2 > 4$. The polymerizability of phenols via HRP catalysis was evaluated by the initial reaction rate.[17] Phenols with electron-donating groups were consumed much faster than those with electron-withdrawing groups. The reaction rate of para- or meta-substituted phenols was larger than that of ortho-substituted ones.

A bienzymatic system was developed as catalyst for the oxidative polymerization of phenols.[18] The HRP-catalyzed polymerization of phenol in the presence of glucose oxidase and glucose gave the polymer in a moderate yield, in which hydrogen peroxide was formed in situ by the oxidative reaction of glucose catalyzed by glucose oxidase.

The morphology of the enzymatically synthesized polyphenols was controlled under the selected reaction conditions. HRP-catalyzed dispersion polymerization of phenol in a mixture of 1,4-dioxane and phosphate buffer using poly(vinyl methyl ether) as stabilizer produced monodisperse polyphenol particles in the submicrometer range.[19] *m*-Cresol and *p*-phenylphenol were also converted to the polyphenol particles by the dispersion polymerization. The particle size could be controlled by the stabilizer concentration and solvent composition. Thermal treatment of these particles afforded uniform carbon particles.

The enzymatic synthesis of polyphenols was carried out not only in the monophasic solvents but in interfacial systems such as micelles, reverse micelles, and biphasic and Langmuir trough systems. *p*-Phenylphenol was polymerized in an aqueous surfactant solution to give the polymer with a narrower molecular weight distribution in comparison with that obtained in the aqueous 1,4-dioxane.[20]

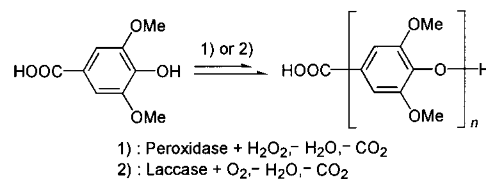
Reverse micellar systems were used for the polymerization of phenol derivatives. HRP-catalyzed polymerization of *p*-ethylphenol in the ternary system composed of a bis(2-ethylhexyl) sodium sulfosuccinate (AOT)–water–isooctane system produced spherical polyphenol particles having 0.1–2 μm diameters quantitatively.[21] Similar particles were obtained by pouring the solution of enzymatically prepared polyphenol into a nonsolvent containing AOT.[22]

The enzymatic polymerization proceeded even in a biphasic system consisting of two mutually immiscible phases (isooctane and water).[23] In the polymerization of *p*-alkylphenols in this system, the molecular weight increased as a function of the carbon number of the alkyl group.

Polyphenol thin films were obtained using Langmuir–Blodgett technique.[21b,24] A monomeric monolayer was formed from *p*-tetradecyloxyphenol and phenol at the air–water interface in a Langmuir trough, which was polymerized by HRP catalyst in the subphase. The polymerized film could be deposited on silicon wafer with a transfer ratio of 100% for the Y-type film with a thickness of 27.8 Å.

Peroxidase-catalyzed polymerization of phenols has provided a new methodology for functional polymeric materials.

Scheme 3



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 T_g (ca. 210 °C) and mechanical toughness. PPO was first prepared from 2,6-dimethylphenol monomer using a copper/amine catalyst system.[25] The HRP-catalyzed polymerization of 2,6-dimethylphenol gave the polymer consisting of exclusively oxy-1,4-phenylene units.[26]

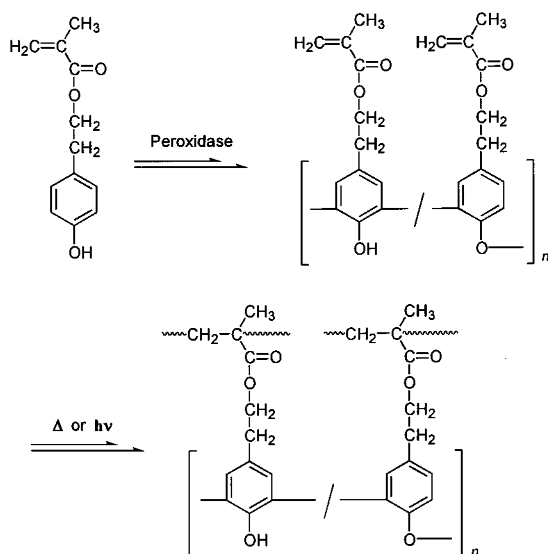
Another PPO derivative was enzymatically obtained from syringic acid (Scheme 3).[27] Both HRP and SBP were active for the polymerization involving elimination of carbon dioxide and hydrogen from the monomer to give the polymer with a molecular weight up to 1.3×10^4. 4-Hydroxy-3,5-dimethylbenzoic acid was also polymerized to give PPO; on the other hand, the polymerization of nonsubstituted 4-hydroxybenzoic acid did not occur under similar reaction conditions. NMR and MALDI-TOF mass analyses showed that the polymer consisted of exclusively 1,4-oxyphenylene units and possessed a phenolic hydroxy group at one terminal end and a benzoic acid group at the other. As one possible application, the polymer 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.[28] The extent of the demethylation was 93%. By utilizing two different terminal functional groups of the polymer, the multiblock copolymer of PPO and aromatic polyester was synthesized by the polycondensation of bisphenol A, isophthalic acid, and the polymer in the presence of triphenylphosphine/hexachloroethane (coupling agent).[29]

Formation of α-hydroxy-ω-hydroxyoligo(oxy-1,4-phenylene)s was observed in the HRP-catalyzed oxidative polymerization of 4,4′-oxybisphenol in aqueous methanol.[30] During the reaction, the redistribution and/or rearrangement of the quinone–ketal intermediate take place, involving the elimination of hydroquinone to give oligo(oxy-1,4-phenylene)s.

Fluorescent naphthol-based polymers were prepared by HRP-catalyzed polymerization of 2-naphthol in AOT/isooctane reverse micelles to give the polymer microspheres.[31] The precipitated polymer was soluble in a range of polar and nonpolar organic solvents and possessed quinonoid structure. The reverse micellar system induced the peroxidase-catalyzed copolymerization of *p*-hydroxythiophenol and *p*-ethylphenol, yielding the thiol-containing polyphenol particles.[32] The attachment of CdS to the particles gave the CdS–polymer nanocomposite showing fluorescence characteristics.

Cross-linkable polyphenols have been enzymatically synthesized. A thermally curable polyphenol

Scheme 4



was synthesized by peroxidase-catalyzed polymerization of bisphenol A.[33] The polymer was cross-linked at 150−200 °C, and the curing improved the thermal stability of the polymer. The reaction with epoxy resin produced the insoluble network polymer. In the HRP-catalyzed polymerization of 4,4′-biphenol, the polymer showing high thermal stability was obtained.[34]

A chemoselective polymerization of a phenol derivative having a methacryloyl group was achieved through HRP catalysis.[35] During the polymerization, the methacryloyl group was not involved to give the polymer having the methacryloyl group in the side chain (Scheme 4). The resulting polymer was readily subjected to thermal and photochemical curings.

HRP-catalyzed polymerizations of hydrophobic monomers, *N*-(4-hydroxyphenyl)maleimide, 4′-hydroxymethacrylanilide, and *N*-methacryloyl-11-aminoundecanoyl-4-hydroxyanilide, proceeded even in water in the presence of 2,6-di-*O*-methylated *β*-cyclodextrin.[36] Formation of water-soluble complexes consisting of the hydrophobic monomers as guest molecules and the cyclodextrin derivative as host was detected. The polymerizable moieties in the polymer were not reacted during the polymerization.

m-Ethynylphenol was chemoselectively polymerized to give the polyphenol having the acetylenic group.[37] For reference, the copper/amine catalyst system induced the selective oxidative coupling of the acetylene group of the monomer to yield the dimer. Thermal treatment of the resulting polymer produced a carbonized polymer in a much higher yield than enzymatically synthesized poly(*m*-cresol).

Cardanol, the 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 component. The SBP-catalyzed polymerization of cardanol in aqueous acetone produced the oily soluble polymer with M_n of several thousands.[38] The carbon−carbon unsaturated group in the side chain of cardanol did not change during the polymerization. The curing by cobalt naphthenate gave the cross-linked film with a high gloss surface. The hydrogenated cardanol derivative was also oxidatively polymerized by HRP.[39]

Scheme 5

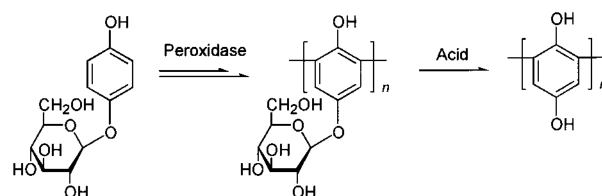


Photo-cross-linkable oligophenols were synthesized by the HRP-catalyzed polymerization of cinnamoylhydroquinone-ester and cinnamoyl-4-hydroxyanilide in an aqueous 1,4-dioxane.[40] UV irradiation of the oligomer films induced the cross-linking by the photochemical [2+2]-cycloaddition of the cinnamoyl function.

A natural phenol, 4-hydroxyphenyl *β*-D-glucopyranoside (arbutin), was subjected to regioselective oxidative polymerization using peroxidase catalyst in a buffer solution, yielding the water-soluble polymer consisting of a 2,6-phenylene unit (Scheme 5).[41] Acidic deglycosylation of the resulting polymer afforded soluble poly(hydroquinone), which may be an *ortho*−*ortho* coupling structure. The resulting polymer was applied as a glucose sensor by utilizing its good redox properties.[42] Chemoenzymatic synthesis of another poly(hydroquinone) was reported; SBP-catalyzed polymerization of 4-hydroxyphenyl benzoate followed by alkaline hydrolysis gave the polymer whose structure was different from that of arbutin.[43]

A thymidine-containing polyphenol was synthesized by SBP-catalyzed oxidative polymerization of thymidine 5′-*p*-hydroxyphenylacetate.[44] Amphiphilic esters of tyrosine were polymerized in a micellar solution to give the polymer showing surface activity at the air−water interface.[45] Polymerization monitoring using a quartz crystal microbalance was reported.

Peroxidase catalyzed the oxidative polymerization of fluorinated phenols to give fluorine-containing polymers.[46] During the polymerization, elimination of fluorine atom partly took place to give the polymer with a complicated structure. Antioxidant effects of the enzymatically synthesized polyphenols were evaluated.[47] The autoxidation of tetralin was significantly suppressed in the presence of the polyphenols.

Enzymatically synthesized polyphenol derivatives are expected to have great potential for electronic applications. The surface resistivity of poly(*p*-phenylphenol) doped with nitrosylhexafluorophosphate was around 10^5 Ω.[4a] The iodine-labeled poly(catechol) showed low electrical conductivity in the range from 10^{-6} to 10^{-9} S/cm.[48] The iodine-doped thin film of poly(phenol-*co*-tetradecyloxyphenol) showed a conductivity of 10^{-2} S/cm, which was much larger than that obtained in aqueous 1,4-dioxane.[24a] The third-order optical nonlinearity (χ^3) of this film was 10^{-9} esu. An order of magnitude increase in the third-order nonlinear optical properties was observed in comparison with that prepared in the aqueous organic solution.

A novel photoactive azopolymer, poly(4-phenylazophenol), was synthesized using HRP catalyst. The polymer exhibited reversible trans to cis photoisomerization of the azobenzene group with a long relaxation time.[49] Hydroquinone mono-oligo(ethylene glycol) ether was polymerized by HRP in aqueous 1,4-dioxane. The film prepared from a mixture of the lithiated polyphenol and poly(ethylene glycol) (PEG) showed high ionic conductivities (4×10^{-5} S/cm).[50] Phenolic copolymers containing fluorophores (fluoroscein and calcein) were synthesized using SBP catalyst and used as an array-based metal-ion sensor.[51] Selectivity and sensitivity of metal sensing were controlled by changing the polymer components. A combinatorial approach for screening of specific metal ions was examined.

In vitro synthesis of lignin, a typical phenolic biopolymer, has been attempted by the HRP-catalyzed terpolymerization of *p*-coumaryl alcohol, coniferyl alcohol, and sinapyl alcohol (14:80:6 mol %) in extremely dilute aqueous solutions at pH 5.5.[52] Dialysis membrane method was applied to the polymerization of coniferyl and sinapyl alcohols, yielding insoluble polymeric materials.[53] Coniferyl alcohol was polymerized by HRP in an aqueous acetone to give the insoluble polymer.[54] In the presence of a small amount of lignin component, the molecular weight distribution became much broader than that in the absence of lignin.[55] HRP catalyzed the polymerization of soluble oligomeric lignin fragments to yield insoluble polymeric precipitates.[56]

Peroxidase-catalyzed grafting of polyphenols on lignin was performed by HRP-catalyzed polymerization of *p*-cresol with lignin in the aqueous 1,4-dioxane or reverse micellar system.[57] Phenol moiety in lignin was reacted with *p*-cresol to produce a lignin−phenol copolymer with a branched and/or cross-linked structure. The product was highly insoluble in common organic solvents.

The peroxidase-catalyzed reaction of low-molecular weight coal (molecular weight $\approx 4 \times 10^3$) was performed in a mixture of DMF and buffer.[58] The resulting product was partly soluble in DMF, and the DMF-soluble part had a larger molecular weight than that of the starting substrate.

Peroxidase catalysis also induced the depolymerization of enzymatically obtained polyphenols.[59] Formation of HRP compound III intermediate may be related to the onset of the depolymerization. Furthermore, the polyphenols were reported to be subjected to biodegradation, although the degradation proceeded slowly.[60]

HRP was used as catalyst for cross-linking of peptides (soy proteins and wheat gliadin).[61] Tyrosine residues of the proteins were subjected to the enzymatic oxidative coupling, yielding a network of peptide chains. The treatment increased the tensile strength of the materials.

Lignin-degradating manganese(II) peroxidase was used as catalyst for the oxidative polymerization of various phenol derivatives such as guaiacol, *o*-cresol, and 2,6-dimethoxyphenol in the aqueous organic solvents.[62]

Model complexes of peroxidase were used as catalysts for the oxidative polymerization of phenols. Hematin, a hydroxyferriprotoporphyrin, catalyzed the polymerization of *p*-ethylphenol in an aqueous DMF.[63] Iron−*N*,*N*′-ethylenebis(salicylideneamine) (Fe−salen) showed high catalytic activity for oxidative polymerization of various phenols.[64] The first synthesis of crystalline fluorinated PPO was achieved by the Fe−salen-catalyzed polymerization of 2,6-difluorophenol. Cardanol was polymerized by Fe−salen to give a cross-linkable polyphenol in high yields.

2. Oxidative Polymerization of Aniline Derivatives

Aniline and its derivatives were oxidatively polymerized by peroxidase catalyst. HRP catalyzed the polymerization of aniline in aqueous organic solvents to produce the polymer with a complicated structure in low yields.[65] The resulting polymer showed good third-order nonlinear optical properties.[66]

The addition of templates enabled the enzymatic production of conducting polymers with well-defined structure.[67] In using sulfonated polystyrene (SPS) as a template, the resulting polymer was soluble in water and the conductivity reached 5×10^{-3} S/cm without doping. Besides SPS, a strong acid surfactant (sodium dodecylbenzenesulfonic acid) or poly(vinylphosphonic acid) provided a suitable local template environment leading to the formation of conducting polyaniline.

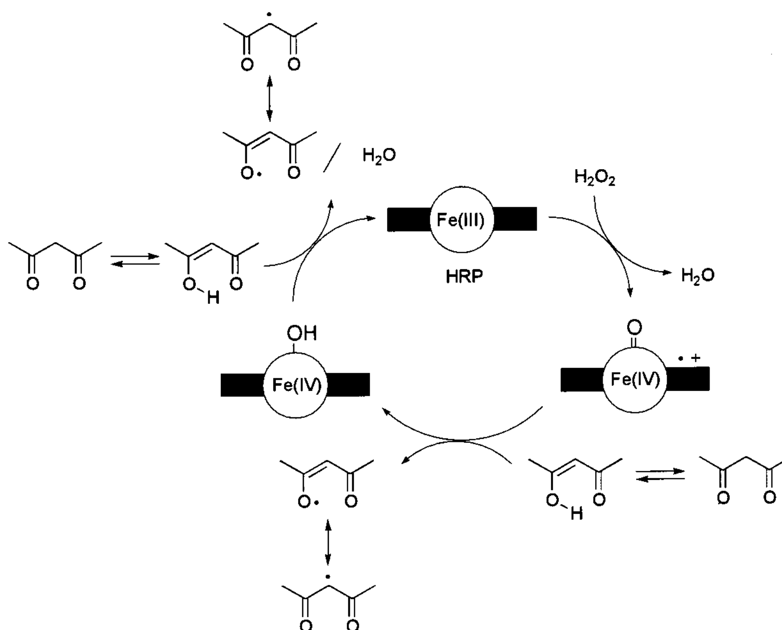
HRP-catalyzed polymerization of *o*-phenylenediamine in an aqueous 1,4-dioxane gave a soluble polymer with a molecular weight of 2×10^4.[68] NMR analysis showed the formation of the polymer consisting of an iminophenylene unit. From *p*- and *m*-isomers, the polymer with well-defined structure was not obtained.[69] HRP-catalyzed polymerization of 4,4′-diaminoazobenzene gave a new photodynamic polyaniline derivative containing an azo group.[70] Various aniline derivatives, *p*-aminobenzoic acid,[71] *p*-aminophenylmethylcarbitol,[72] 2,5-diaminobenzenesulfonate,[73] and *p*-aminochalcones,[74] were polymerized by peroxidase catalyst. Monolayer of aniline/*p*-hexadecylaniline prepared by LB technique at the air−water interface was polymerized through HRP catalysis to give polymeric monolayer.[24]

A new class of polyaromatics was synthesized by peroxidase-catalyzed oxidative copolymerization of phenol derivatives with anilines. In the case of a combination of phenol and *o*-pheneylenediamine, FT-IR analysis showed the formation of the corresponding copolymer.[75]

3. Polymerization of Vinyl Monomers

Some oxidoreductases have been reported to induce polymerization of vinyl monomers. A novel initiating system for vinyl polymerization, HRP/hydrogen peroxide/β-diketones such as acetylacetone, was demonstrated in which the catalytic action of HRP generates carbon radical, a real initiating species, from hydrogen peroxide and β-diketone through an oxidoreductive pathway (Scheme 6).[76] Hydrophobic monomers, styrene[77] and methyl methacrylate (MMA),[78] were also polymerized by this initiating

Scheme 6



system in a mixture of water and tetrahydrofuran. The polymer from MMA had syndiotactic diad fractions ranging from 0.82 to 0.87.[78] Manganese peroxidase catalyzed the acrylamide polymerization in the presence of acetylacetone.[79]

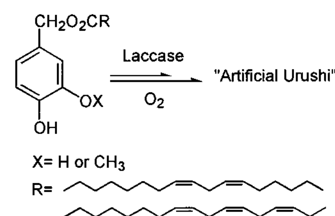
B. Laccases

Laccases having a Cu active site catalyze the oxidative coupling of phenols. Laccases derived from *Pycnoporus coccineus* (PCL) and *Myceliophthore* were active for the polymerization of syringic acid to give PPO with a molecular weight up to 1.8×10^4 (Scheme 2).[27,80] Enzymatic synthesis of PPO was also achieved from 2,6-dimethylphenol using PCL catalyst.[26] The polymerization of 1-naphthol using laccase from *Trametes versicolor* (TVL) proceeded in aqueous acetone to give the polymer with a molecular weight of several thousands.[81]

Coniferyl alcohol was polymerized by laccase catalyst. The polymerization behavior depended on the origin of the enzyme. PCL and laccase from *Coriolus versicolar* showed high catalytic activity to give the dehydrogenative insoluble polymer, whereas very low catalytic activity was observed in laccase from *Rhus vernicifera* Stokes.[54] The increase of the molecular weight was observed in the treatment of soluble lignin using TVL catalyst.[82]

Urushi is a Japanese traditional coating showing excellent toughness and brilliance for a long period. 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 the 3- or 4-position of catechol.[83] Film-forming of urushiols proceeds under air at room temperature without organic solvents; hence, urushi seems to be very desirable for coating materials from an environmental standpoint. In vitro enzymatic hardening reaction of catechol derivatives bearing an unsaturated alkenyl group at the 4-position of the

Scheme 7



catechol ring proceeded using PCL catalyst to give the cross-linked film showing excellent dynamic viscoelasticity.[84]

A novel system of enzymatic polymerization, i.e., a laccase-catalyzed cross-linking reaction of new urushiol analogues for the preparation of "artificial urushi", was demonstrated (Scheme 7).[85] Single-step synthesis of the urushiol analogues having ester group was achieved by using lipase as catalyst. These compounds were cured in the presence of laccase catalyst under mild reaction conditions without use of organic solvents to produce the cross-linked polymeric film. Properties of the film are comparable to those of natural urushi with a high gloss surface and dynamic viscoelasticity (Figure 1).

Laccase catalyzed the polymerization of acrylamide in water.[86] The high molecular weight polymer was

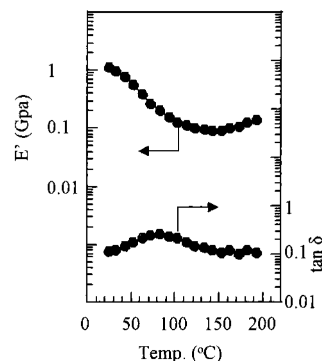


Figure 1. Dynamic viscoelasticity of artificial urushi from urushiol analogue having a linolenic acid group.

obtained using PCL catalyst at 50−80 °C. The polymerization in the presence of acetylacetone proceeded at room temperature. A combination of laccase and organic peroxide initiated the polymerization of acrylamide in the presence of lignin, yielding lignin-*graft*-polyacrylamide.[87]

C. Other Oxidoreductases

Bilirubin oxidase (BOD), a copper-containing oxidoreductase, catalyzes the oxidative polymerization of aniline and 1,5-dihydroxynaphthalene. The polymerization of aniline in a buffer solution in the presence of BOD-adsorbed solid matrix gave the polyaniline film containing the active enzyme.[88] The film was electrochemically reversible in its redox properties in acidic aqueous solution. In the BOD-catalyzed polymerization of 1,5-dihydroxynaphthalene in aqueous organic solvents, the insoluble polymer was formed.[89] The polymer films exhibited a wide band from 300 to 470 nm in their UV spectrum, indicating the formation of a long π-conjugated structure. The polymer treated with $HClO_4$ showed an electroconductivity of 10^{-3} S/cm.

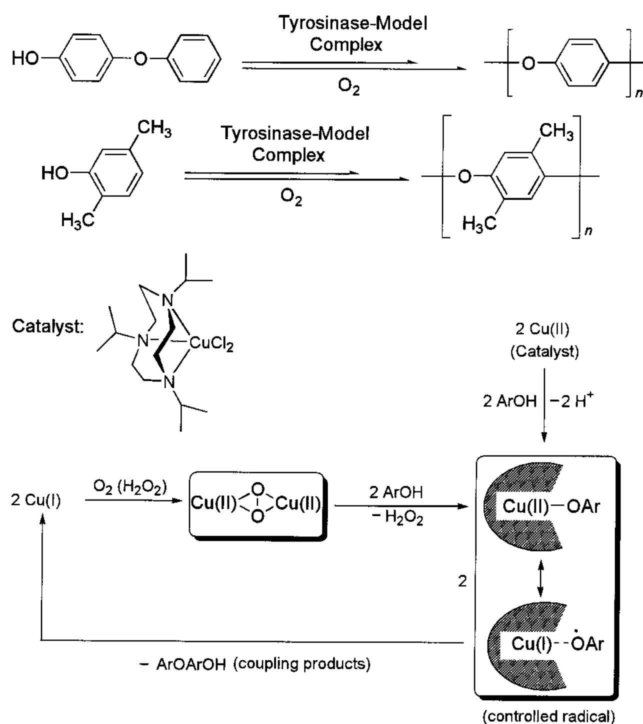
Tyrosinase (polyphenol oxidase, a copper-containing monooxygenation enzyme) was used as catalyst for the modification of natural polymers. Phenol moiety-incorporated chitosan derivatives were subjected to tyrosinase-catalyzed cross-linking, yielding stable and self-sustaining gels.[90] Tyrosinase also catalyzed the hybrid production between the modified chitosan and proteins.

The enzymatic treatment of chitosan in the presence of tyrosinase and phenol derivatives produced new materials based on chitosan.[91] During the reaction, unstable *o*-quinones were formed, followed by the reaction with the amino group of chitosan to give the modified chitosan. The tyrosinase-catalyzed modification of chitosan with phenols dramatically altered rheological and surface properties of chitosan. The modification with chlorogenic acid onto chitosan conferred the water solubility of chitosan under basic conditions.[92] A new water-resistant adhesive was developed by the tyrosinase-catalyzed reaction of 3,4-dihydroxyphenethylamine and chitosan.[93] Poly(4-hydroxystyrene) was modified with aniline by using tyrosinase catalyst.[94] The incorporated ratio of aniline into the polymer was very low (1.3%).

Tyrosinase catalyzed the oxidative coupling of soluble lignin fragments to give the insoluble polymer.[56] Tyrosinase model complexes catalyzed the regioselective oxidative polymerization of phenols, leading to the formation of aromatic polyethers with well-defined structure.[95−97] The reaction at the unsubstituted ortho positions did not take place, in sharp contrast to the polymerization by a Cu−amine catalyst system.[25] From 4-phenoxyphenol[95] and 2,5-dimethylphenol,[96] crystalline PPO derivatives were formed (Scheme 8). The polymer from the latter monomer possessed a melting point higher than 300 °C, a class of super engineering plastics.[96] A "radical-controlled" reaction mechanism is proposed for the high regioselectivity.

Glucose oxidase initiated the vinyl polymerization in the presence of Fe^{2+} and dissolved oxygen.[98] The

Scheme 8



formation of cross-linked product was observed in the polymerization of acrylamide in the presence of bisacrylamide catalyzed by xanthine oxidase, chloroperoxidase, and alcohol oxidase.[99]

III. Transferases

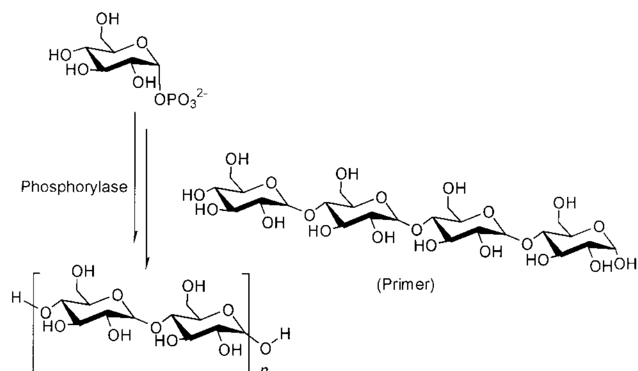
Transferases are enzymes transferring a group from one compound (generally regarded as donor) to another compound (generally regarded as acceptor). For example, a glycosyl group as donor is transferred to an alcohol as acceptor to form a glycosidic bond and an acyl group as donor to an alcohol as acceptor giving rise to an ester bond. Several transferases such as phosphorylases and synthases have been found to be effective for catalyzing in vitro synthesis of polysaccharides and polyesters. It is to be noted, however, that some of the in vitro reactions below, such as cellulose, chitin, hyaluronic acid, and polyester syntheses, catalyzed by the respective synthases are occurring in a manner similar to biosynthetic pathways in vivo. Such reactions are then not in the category of enzymatic polymerization in a strict sense of its definition but are probably instructive to readers.

A. Glycosyltransferases

1. Phosphorylases

A phosphorylase catalyzes in vitro production of amylose (poly-α(1→4)-D-glucopyranose) and its derivatives. Amylose was synthesized from D-glucosyl phosphate as a substrate monomer and malto-oligomers with a minimum length of four glucosyl residues as a primer by using potato phosphorylase (Scheme 9).[100] The reaction proceeded analogously to a living polymerization to form amyloses having relatively uniform chain lengths. Branched polymers

Scheme 9



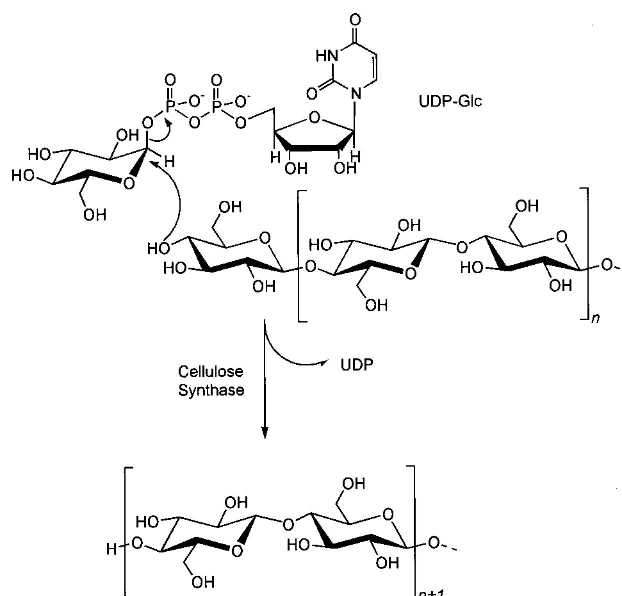
of star and comb shapes carrying amylose chains of uniform length were also obtained by using potato phosphorylase.[101] Poly(dimethylsiloxane-*graft*-α(1→4)-glucopyranose) was synthesized from D-glucosyl phosphate and a polyinitiator based on polysiloxane with statistically distributed maltoheptaonamide and maltoheptaoside by using potato phosphorylase.[102] Similarly, this method was extended widely to prepare styryl-type amylose macromonomer,[103] amylose-*graft*-poly(L-glutamic acid),[104] amylose-*block*-polystyrene,[105] amylose-*block*-poly(ethylene oxide),[106] and amylose-containing silica gel.[107]

The synthesis of 2-deoxyamylose derivatives by enzymatic chain elongation of glycogen was reported; only an average of up to 1.5 units of 3- and 4-deoxyglucosyl phosphate were transferred by phosphorylase.[108] On the other hand, an average of 20 units of 2-deoxyglucosyl units were connected to maltotetraose by using potato phosphorylase.[109] One day incubation of D-glucal and the tetrasaccharide with phosphorylase and 0.1 equiv of inorganic phosphate yielded a precipitate of corresponding polysaccharide. The tetrasaccharide is the shortest length as a primer required for the phosphorylase reaction. The polysaccharide became insoluble at a chain length of about 20 and was then released from the enzymatic glycosylation process.

The phosphorolytic synthesis of cello-oligosaccharides by cello-oligosaccharide phosphorylase was carried out by using various cellobiosyl residues as glycosyl acceptors and α-D-glucopyranosyl phosphate as glycosyl donor.[110] The crystalline precipitate was obtained, which showed the diffraction diagrams of low molecular weight cellulose II. NMR measurement revealed that an average degree of polymerization of the cello-oligosaccharides was about 8. Cello-oligosaccharides beyond this chain length become insoluble in water. Subsequent chain elongation in the phosphorolytic synthesis may be possible as long as the product is soluble. The product is therefore considered to dissociate completely from the enzyme after each addition of a glucose unit. Various cello-oligosaccharide derivatives substituted at their reducing end were also obtained by the phosphorolytic synthesis in the presence of various primers.

In all cases using phosphorylase as catalyst, it is required to employ a large excess amount of a substrate monomer (glycosyl donor) for a primer in order to shift the reaction equilibrium to the polymer formation.

Scheme 10



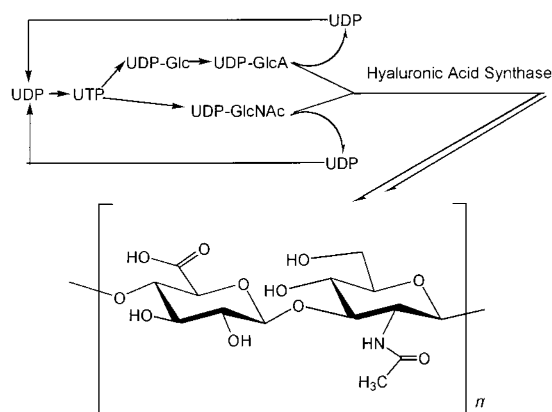
The molecular mechanism of the substrate recognition and phosphorolysis of phosphorylase was presented.[111] The ternary complex of malto-oligosaccharide phosphorylase, 4-*S*-α-D-glucopyranosyl-4-thiomaltotetraose, and phosphate shows that the phosphate group attacked the glycosidic linkage and promoted the phosphorolysis.

2. Glycosyl Transferases

Biosynthetic mechanisms of cellulose and chitin in living cells have been extensively studied; cellulose and chitin were also formed in vitro from the activated monomers, uridine diphosphate glucose (UDP-Glc) and UDP-*N*-acetyl-glucosamine (UDP-GlcNAc), catalyzed by cellulose and chitin synthases, respectively.[112] Glycosyltransferases of the Leloir pathway use individual sugar nucleotides as donors, which are activated as glycosyl esters of nucleoside mono- or diphosphates.[113] Scission of the phosphoester bond between uridine diphosphate (UDP) and the monosaccharide will supply the free energy that drives glycosidic linkage formation. A single displacement mechanism with inversion of configuration to yield β-1,4-linkages has been proposed, where the nucleophilic C4−OH at the nonreducing chain end attacks the α-C1-position of UDP-Glc. The reducing chain end points away from the catalytic enzyme (Scheme 10). On the other hand, glycogen synthase forms α-linked products from α-linked donor substrates with retention of anomeric carbon configuration at the reaction center.[114] The "retaining" mechanism is most likely due to the formation of a glycosyl enzyme intermediate in the enzymatic process.

Even though the relatively high cost of the glycosyl donors and limited enzyme availability are drawbacks of using glycosyl transferases to saccharide synthesis, the modern recombinant DNA technology enables the increasing availability of glycosyl transferases. Furthermore, the method appears to be effective even for a large-scale stereocontrolled oligosaccharide synthesis when it is combined with in situ regeneration of sugar nucleotides.[115] This strat-

Scheme 11



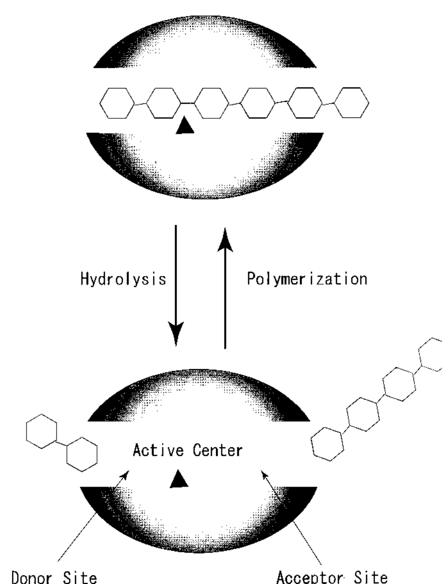
egy also avoids the problem of product inhibition by released nucleoside mono- or diphosphates. Sugar nucleotides are regenerated via sugar nucleotide pyrophosphorylases or sugar nucleotide synthases with a catalytic amount of nucleoside mono- or diphosphate and a stoichiometric amount of the donor monosaccharide. For example, hyaluronic acid with a molecular weight of about 5.5×10^5 has been synthesized from UDP-GlcNAc and UDP-glucronic acid (UDP-GlcA) using hyaluronic acid synthase coupled with regeneration of the sugar nucleotides (90% yield, Scheme 11).[116] For this enzymatic preparation of hyaluronic acid, seven kinds of enzymes, hyaluronic acid synthase, UDP-Glc dehydrogenase, UDP-Glc pyrophosphorylase, UDP-GlcNAc pyrophosphorylase, pyruvate kinase, lactate dehyrogenase, and inorganic pyrophosphatase, were used together in a polymerization solution.

Chitin oligosaccharides were obtained by a bacterial chitin oligosaccharide synthase (NodC) using UDP-GlcNAc as a donor. Elongation of the growing chitin oligosaccharide chain by NodC proceeds by the addition of monosaccharides to C4−OH of the nonreducing terminal GlcNAc residue. Chitin oligosaccharide synthesis by NodC is considered to occur by a processive mechanism because chitin oligosaccharides ranging from chitobiose to chitotetraose could not act as efficient primers for NodC. Therefore, successive addition of saccharide units to a growing chain only proceeds while the product chain remains in contact with the enzyme.[117]

B. Acyltransferases

Poly(β-hydroxybutyrate) (PHB) is accumulated within the cells of a wide variety of bacteria as an intracellular energy and carbon storage material. Poly(hydroxyalkanoate)s (PHA)s are commercially produced as biodegradable plastics. In the biosynthetic pathways of PHA, the last step is the chain growth polymerization of hydroxyalkanoate CoA esters catalyzed by PHA polymerase (synthase). In the present review, we classified PHA polymerases into transferases by taking them as a family of acyltransferases. PHA polymerase from *Ralstonia eutropha* polymerized the CoA monomers of (*R*)-hydroxyalkanoate in vitro to give the high molecular weight homopolymers and copolymers with well-defined structure.[118] The molecular weight of the

Scheme 12



polymers was found to be inversely proportional to the molar ratio of monomer-to-enzyme, and the obtained polymer had a narrow molecular weight distribution, suggesting that the polymerization proceeds in a living fashion. Random copolymers were obtained from the mixture of the two CoA esters in the presence of the polymerase, whereas the sequential copolymerization produced the block copolymers.

In the combination of recombinant PHA synthase from *Chromatium vinosum* with propionyl-CoA transferase of *Clostridium propionicum*, a two-enzyme in vitro PHB biosynthesis system from (*R*)-hydroxybutyric acid was demonstrated.[119] This system required only catalytic amounts of expensive CoA, and hence, PHA could be readily produced in a semipreparative scale.

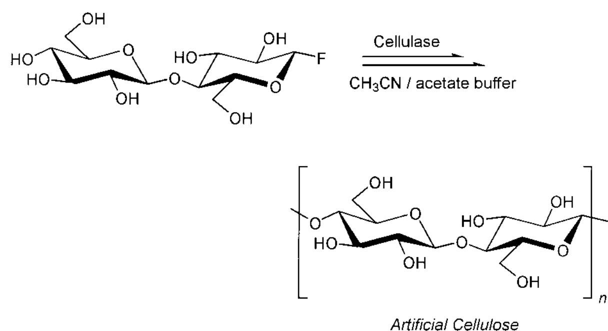
IV. Hydrolases

It is generally accepted that an enzymatic reaction is virtually reversible, and hence, the equilibrium can be controlled by appropriately selecting the reaction conditions. On the basis of this view, many hydrolases, which are enzymes catalyzing a bond-cleavage reaction by hydrolysis, have been employed as catalysts for the reverse reaction of hydrolysis, leading to polymer production by a bond-forming reaction.

A. Glycosidases

Glycosidases are hydrolysis enzymes to cleave the glycosidic linkage of glycan chains with water. Their catalytic center is normally surrounded by donor and acceptor sites where the reducing and nonreducing sites, respectively, of glycan chains are involved in both reactions of the hydrolysis (bond-cleavage) and the reverse reaction (bond-forming) (Scheme 12). Two major types of glycosidases are known; exo-glycosidases cleaving external glycosidic linkages of polysaccharide chains to liberate mono, di-, or oligo-saccharides from the nonreducing terminal and endo-glycosidases randomly cleaving internal glycosidic linkages of the chains.

Scheme 13



1. Natural Glycosidases

Glycosidase-catalyzed in vitro polymerizations enabled the synthesis of not only various natural polysaccharides, but also some unnatural polysaccharides, when the substrate monomer is appropriately designed in combination with a selected enzyme.

a. Synthesis of Natural Polysaccharides. There are two main approaches for glycosidase-catalyzed synthesis of glycosidic-linked compounds: direct reversal of hydrolysis (equilibrium-controlled synthesis) and promoted formation of a glycosyl enzyme intermediate (kinetically controlled synthesis).[120] The former synthesis uses a high concentration of mono- or oligo-saccharide as a donor, addition of organic cosolvents, and elevated reaction temperatures to achieve significant transformation. On the other hand, the latter synthesis needs activated starting substrates such as glycosyl fluorides or aryl glycosides as donors. The whole point of the formation of a glycosidic linkage by glycosidases is a rapid formation of an activated glycosyl enzyme intermediate and its rapid reaction with the glycosyl acceptor, which should be much easier than with water.

A typical example of the equilibrium-controlled synthesis is the preparation of hexa-*N*-acetylchitohexaose and hepta-*N*-acetylchitoheptaose from di-*N*-acetylchitobiose under the conditions of high substrate concentration (10 wt %), high ionic strength (30 wt % ammonium sulfate), and high temperature at 70 °C by using egg yolk lysozyme (exo-glycosidase).[121] These products are practically insoluble in high salt aqueous solution to be selectively precipitated out.[122]

Cellulase was found to be effective in the synthesis of artificial cellulose in a single-step reaction by polycondensation of β-D-cellobiosyl fluoride (Scheme 13).[123] The polymerization is a repetition of the transglycosylation reaction, which became predominant over the hydrolysis reaction when the enzymatic polycondensation was carried out in a mixed solvent of acetonitrile/acetate buffer (5:1, pH 5). This synthesis is therefore kinetically controlled as well as equilibrium controlled. The β configuration of the C1 fluorine atom is necessary to form a reactive intermediate leading to a β(1→4) product via a "double displacement mechanism".[124] Thus, this method provided the first successful in vitro synthesis of cellulose, the most abundant biomacromolecules on the earth, the synthesis of which had been unsolved for one-half a century.[123]

Artificial cellulose showed the cellulose II allomorph, a thermodynamically more stable form with an antiparallel structure, by X-ray diffraction study, when a crude celluase was employed for the enzymatic polymerization.[123] The other allomorph cellulose I is a thermodynamically metastable form with a parallel structure, which living cells normally produce, but was believed impossible to be realized in vitro. Interestingly, however, the in vitro synthesis of cellulose I was successfully achieved by using a purified cellulase.[125] The molecular packing of glucan chains in a crystal is affected by the purity of the enzyme as well as the enzymatic polymerization conditions. A novel concept "choroselectivity" was therefore proposed, which is concerned with the intermolecular relationship in packing of polymers having directionality in their chains.[126]

Furthermore, the enzymatic polymerization provided a novel three-dimensional spherulite composed of artificial cellulose II (Figure 2).[127] The spherulites consisted of single crystals with the glucan chains oriented perpendicular to the crystalline plane, which is entirely different from those obtained from bacterial cellulose.[128]

Xylan was prepared by enzymatic polycondensation similar to the case of artificial cellulose.[129] β-Xylo-

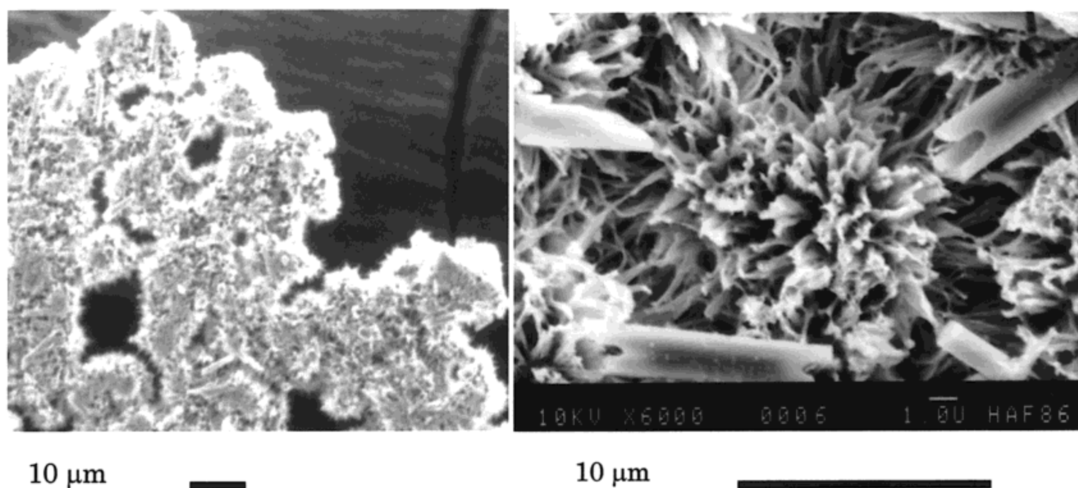
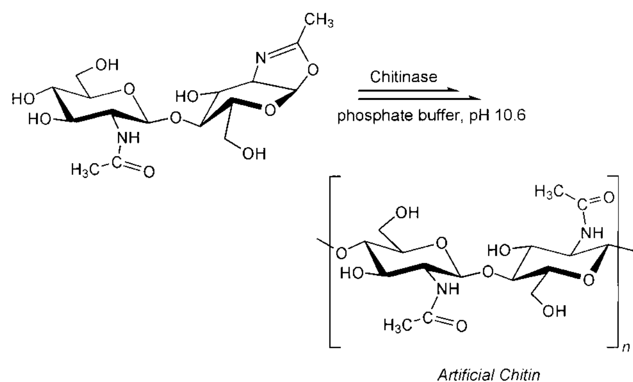


Figure 2. SEM observations of a plate of artificial cellulose spherulites.

Scheme 14



biosyl fluoride was used as a substrate monomer for crude cellulase containing xylanase in a mixed solvent of acetonitrile and acetate buffer. The reaction should proceed through the formation of a glycosyl–enzyme intermediate or a glycosyl oxocarbenium ion intermediate at an active site of the enzyme, followed by the attack of the 4-hydroxy group of an acceptor molecule.

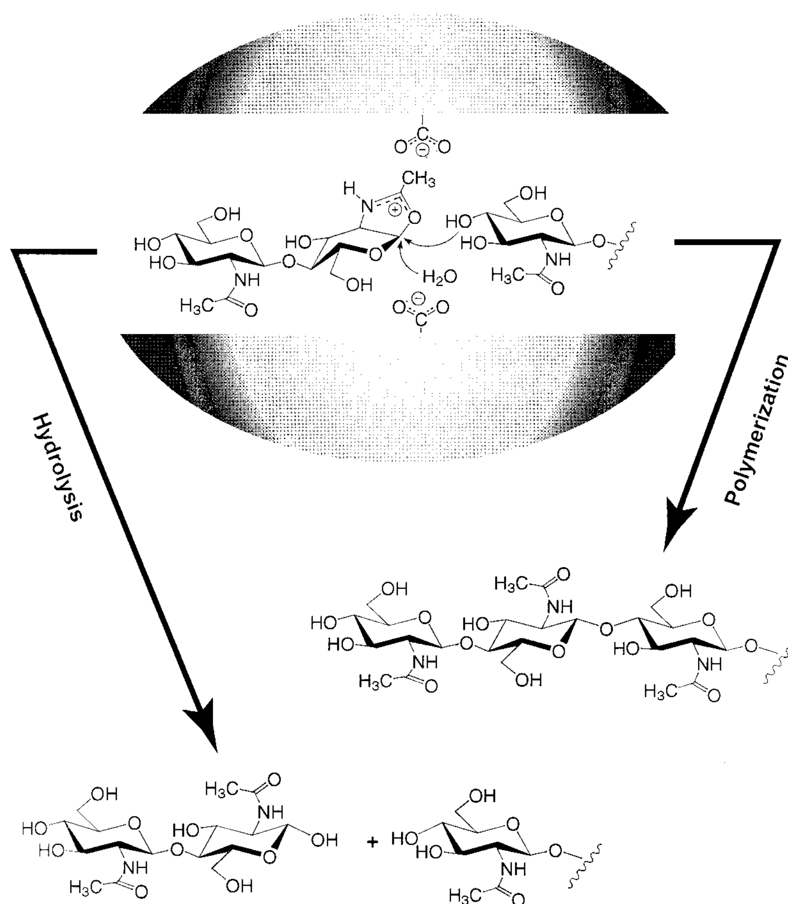
Maltooligosaccharides (artificial amyloses) were prepared by polycondensation of α-D-maltosyl fluoride using α-amylase as the catalyst in a mixed solvent of methanol–phosphate buffer (pH 7).[130] The yielded maltooligosaccharides contained a mixture from triose to heptaose. The formation of the odd-numbered maltooligosaccharides may be due to enzymatic hydrolysis of the products during the reaction. The formation of α(1→4)-glycosidic linkages is explained by a mechanism involving "double displacement" of the C1 carbon configuration of the monomer.

Chitin is the most abundant biomacromolecule in the animal field, which is found normally in invertebrates as a structural component. This important polysaccharide was synthesized for the first time by the enzymatic polymerization using chitinase and a chitobiose oxazoline derivative (Scheme 14).[131] The latter activated monomer has a distorted structure with an α configuration at C1, which resembles a transition-state structure of substrate chitin at the active site during a hydrolysis process (Scheme 15).[3b,131,132] The ring-opening polyaddition of the chitobiose oxazoline derivative was exclusively promoted by chitinase at pH 10.6, where the hydrolytic activity of chitinase was very much lowered.

Upon enzymatic polymerization to produce the artificial chitin, spherulites of 20–50 μm in diameter were also obtained (Figure 3).[133] Platelike single crystals were gradually shaped into ribbons, followed by formation of bundlelike assemblies to grow into spherulites in the polymerization solution.

Hyaluronidase is one of the glycosidases catalyzing hydrolysis of glycosidic linkages of hyaluronic acid (HA). It is known that the enzyme also catalyzes the transglycosylation reaction. By utilizing both catalysis functions, reaction between HA (molecular weight $\approx 8 \times 10^5$) as a donor and HA hexasaccharide having glucuronic acid at the nonreducing terminal as an acceptor took place to reconstruct oligomers (up to

Scheme 15



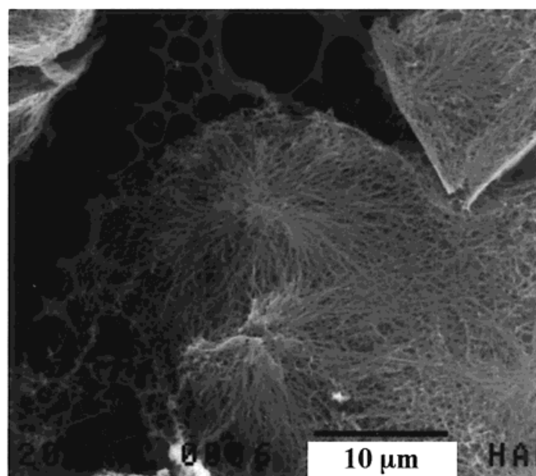
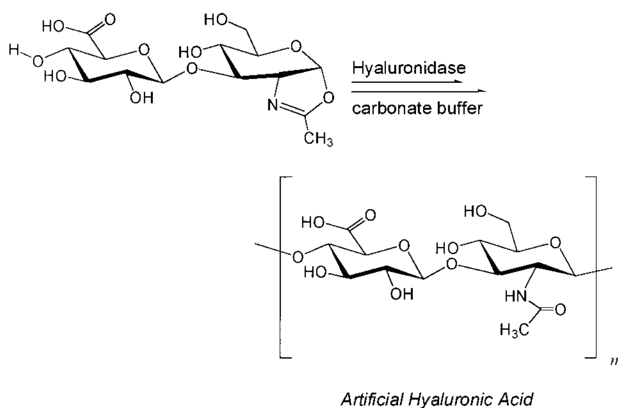


Figure 3. Electron micrograph of artificial chitin spherulite by SEM.

Scheme 16



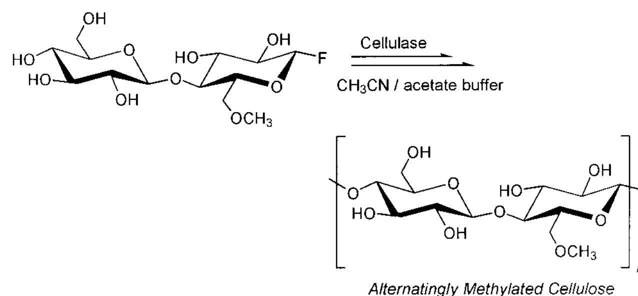
22mers) of HA. During the reaction, a disaccharide unit was released via hydrolysis from the HA and transferred to the nonreducing terminal of the acceptor.[134]

A new enzymatic polymerization has been found to produce artificial HA via ring-opening addition polymerization catalyzed by hyaluronidase (Scheme 16).[135] The oxazoline part of the starting substrate monomer is a latent *N*-acetyl group of an *N*-acetylglucosamine unit which acts as a donor, adding to 4-OH of glucuronic acid at the acceptor site. This polymerization gave HA having a perfect structure containing GlcNAcβ(1→4)GlcUAβ(1→3) linkages with a molecular weight around 20 000.

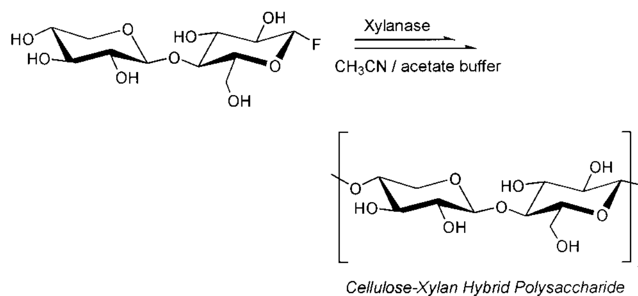
b. Synthesis of Unnatural Polysaccharides. Unnatural polysaccharides were also obtained by the enzymatic polymerization. 6-*O*-Methyl-β-cellobiosyl fluoride was used as a substrate of cellulase, giving rise to an alternatingly 6-*O*-methylated cellulose derivative (Scheme 17).[136,137] Another cellobiose derivative, 6′-*O*-methyl-β-cellobiosyl fluoride, gave only a mixture of low molecular weight oligomers.[137] The difference of reactivity between the two monomers may be explained by easier acceptance of the 6-*O*-methyl glucose unit as the nonreducing terminal at the acceptor site.

The enzymatic polymerization method was extended to use disaccharide monomers composed of different kinds of monosaccharide units to obtain

Scheme 17



Scheme 18



unnatural hybrid polysaccharides. β-Xylopyranosyl−glucopyranosyl fluoride was used as a substrate monomer of xylanase, giving rise to a cellulose−xylan hybrid polymer (Scheme 18).[138]

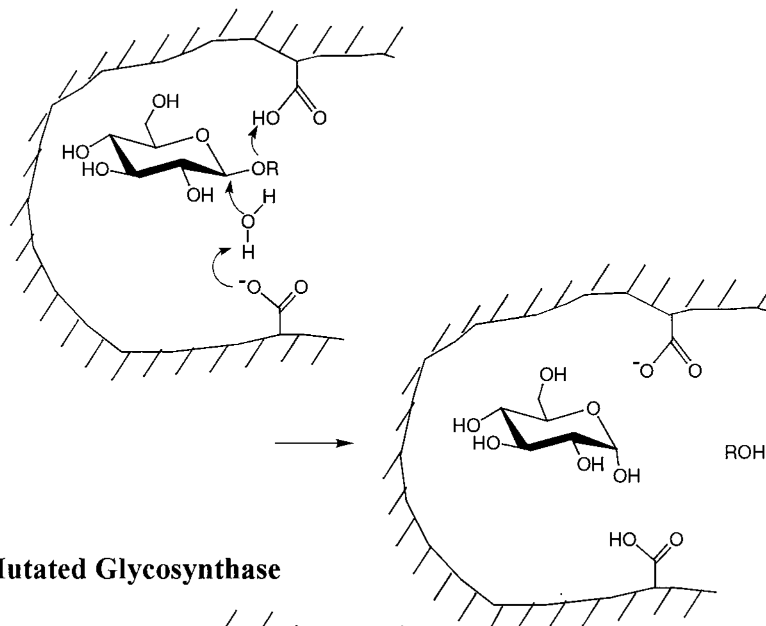
Thiooligosaccharides can be inhibitors of cellulases and are useful for elucidation of the molecular mechanism of the cellulolytic actions. Hemithiocello-oligosaccharides from tetraose to tetradecaose were synthesized by a cellulase mixture using 4-thio-β-cellobiosyl fluoride as an activated donor in a buffer/acetonitrile solvent system.[139]

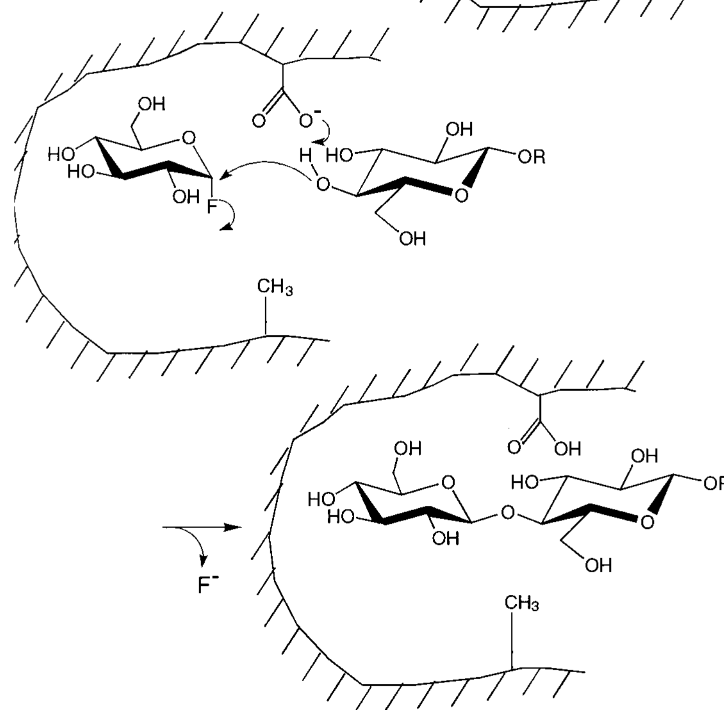
2. Mutated Glycosidases

Novel catalysts by mutating glycosidases to abolish glycosyl hydrolase activity toward unactivated glycosides were developed.[140] The active site of a glycosidase is typically equipped with two residues having a carboxylate side chain to facilitate glycosidic linkage hydrolysis. Conversion of one of the carboxylates to an alanine should make the mutant inactive toward *O*-glycosidic linkages due to the lack of a key catalytic residue. However, the active site may retain the correct steric environment for the formation of a reactive glycosyl donor (Scheme 19). Therefore, this mutated glycosidase may catalyze the ligation of an activated α-glycosyl derivative, bound at the active site in place of the normal glycosyl−enzyme intermediate, to a suitable acceptor sugar bound in the aglycon pocket but does not hydrolyze them.[140,141] Indeed, the mutated β-glycosidase where Glu358 was replaced with Ala formed a tetrasaccharide from 2 equiv of α-glucosyl fluoride and *p*-nitrophenyl cellobioside in a 64% yield.[140] This mutant was, therefore, named as “glycosynthase”.

This methodology was applied to the retaining cellulase endoglucanase I (Cel7B).[142] The catalytic nucleophile Glu197 was replaced with Ala to abolish the hydrolytic activity. The mutated endoglucanase catalyzed highly efficient synthesis of β(1→4)-oligo- and polysaccharides in combination with α-glycobio-

Scheme 19





syl fluorides including 6-substituted derivatives as activated donors. The acceptor subsite of the mutant of Cel7B accommodated various mono- and disaccharides. Notably, the polymerization occurred in a phosphate buffer (pH 7.0).

T4 lysozyme belongs to family 19 chitinases, which show inversion of the anomeric configuration according to the single displacement hydrolysis mechanism.[143] Thr26 is located at the α side of the substrate and functions as a general acid in cooperation with carboxylic acid nearby. The substitution of Thr26 with His converted the lysozyme from an inverting to a retaining enzyme.[144] This altering can be explained by the nucleophilic property of His to yield a glycosyl enzyme intermediate. Upon point mutation, the mutant showed transglycosylation activity, which was notably more effective than hydrolysis activity.[145]

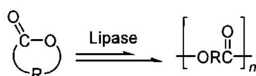
All these instances show the possibility that glycosidases can be converted to glycosynthase by single substitution at the active site, which suppresses the hydrolysis activity of glycosidases. At the same time, however, the nucleophilic attack of aglycon at the acceptor site to the anomer carbon should be significantly promoted in these mutants. This molecular mechanism remains to be solved.

B. Lipases

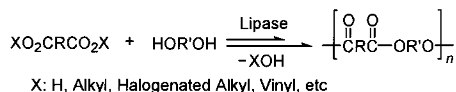
Lipase is an enzyme which catalyzes the hydrolysis of fatty acid esters normally in an aqueous environment in living systems. On the other hand, some lipases are stable in organic solvents and can be used

Scheme 20

Ring-Opening Polymerization of Lactones

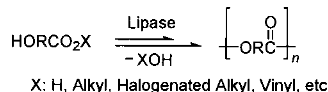


Polycondensation of Dicarboxylic Acids or Their Derivatives with Glycols



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

Polycondensation of Oxyacids or Their Esters



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

as catalyst for esterifications and transesterifications.[146] This specific catalysis enabled production of useful polyesters and polycarbonates by various polymerization modes.[147] Typical reaction types of lipase-catalyzed polymerization leading to polyesters are summarized in Scheme 20.

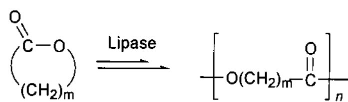
1. Ring-Opening Polymerization of Cyclic Monomers

The first report on an enzymatic ring-opening polymerization appeared in 1993;[148a,149] ϵ-caprolactone (ϵ-CL, seven-membered lactone) was polymerized using lipase as catalyst. Thereafter, various cyclic compounds were subjected to ring-opening polymerization mainly by lipase catalyst. Figure 4 summarizes cyclic monomers polymerized by enzymes. Among them, the lipase-catalyzed polymerization of lactones has been most extensively investigated.

So far, nonsubstituted lactones with a ring size from 4 to 17 were polymerized by lipase catalyst to give the corresponding polyesters (Scheme 21). *Candida rugosa* lipase (lipase CR) catalyzed the polymerization of β-propiolactone (β-PL, four-membered) to give a polymer with high molecular weight (weight-average molecular weight (M_w) > 5×10^4).[150] The polymerization of β-PL by *Pseudomonas* family lipases produced a mixture of linear and cyclic oligomers with a molecular weight of several hundreds.[151]

PHB was enzymatically synthesized from β-butyrolactone (β-BL).[152] Even at a high temperature (100 °C), porcine pancreas lipase (PPL) and lipase CR acted as catalyst to give PHB with M_w up to 7300.

Scheme 21



m=2 (4-Membered) : β-PL
m=3 (5-Membered) : γ-BL
m=4 (6-Membered) : δ-VL
m=5 (7-Membered) : ε-CL
m=7 (9-Membered) : OL
m=10 (12-Membered) : UDL
m=11 (13-Membered) : DDL
m=14 (16-Membered) : PDL
m=15 (17-Membered) : HDL

The resulting products contained a significant amount of cyclic oligo(β-hydroxybutyrate)s, which were formed by the lipase-catalyzed intramolecular cyclization of PHB.[153]

An enantioselective polymerization of four-membered lactones was demonstrated. Racemic α-methyl-β-propiolactone was stereoselectively polymerized by *Pseudomonas cepacia* lipase (lipase PC) to give an optically active (*S*)-enriched polyester with enantiomeric excess (ee) of 50%.[154] From racemic β-BL, (*R*)-enriched PHB with 20–37% ee was formed by using thermophilic lipase as catalyst.[155]

The chemoenzymatic synthesis of biodegradable poly(malic acid) was achieved by the lipase-catalyzed polymerization of benzyl β-malolactonate, followed by the debenzylation.[156] The molecular weight of poly(benzyl β-malolactonate) increased by the copolymerization with a small amount of β-PL using lipase CR catalyst.

Five-membered unsubstituted lactone, γ-butyrolactone (γ-BL), is not polymerized by conventional chemical catalysts. However, oligomer formation from γ-BL was observed by using PPL or *Pseudomonas* sp. lipase as catalyst.[152a,157] δ-Valerolactone (δ-VL, six-membered) was polymerized by various lipases of different origin to give the polymer with M_n of several thousands.[148] Another six-membered lactone, 1,4-dioxan-2-one, was polymerized by *Candida antarctica* lipase (lipase CA) to give the polymer with M_w higher than 4×10^4.[158] The resulting polymer is expected as a metal-free polymeric material for medical applications.

As for the enzymatic ring-opening polymerization of ϵ-CL, various commercially available lipases have been tested as a catalyst. Several crude lipases (PPL, lipases CR, PC, and *Pseudomonas fluorescens* lipase (lipase PF)) induced the polymerization; however, a

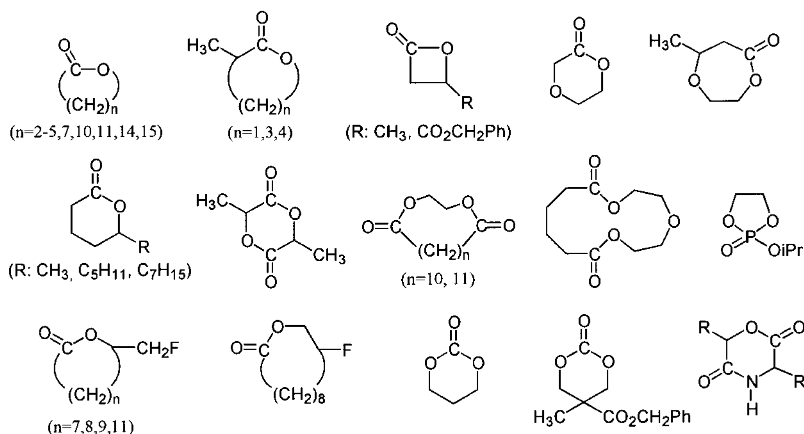


Figure 4. Cyclic monomers polymerized by lipases.

relatively large amount of catalyst (often more than 40 wt % for ϵ-CL) was required for the efficient production of the polymer.[148,159] On the other hand, lipase CA showed high catalytic activity toward the ϵ-CL polymerization; a very small amount of lipase CA (less than 1 wt % for ϵ-CL) was enough to induce the polymerization.[160] The polymer structure depended on the reaction conditions; the polymerization in bulk produced the linear polymer, whereas the main product obtained in organic solvents was of cyclic structure.[161]

In the case of polymerization in organic solvents, the solvent hydrophobicity greatly affected the polymerization behaviors. In the lipase CA-catalyzed polymerization, the efficient production of the polymer was achieved in solvents having log P (P = partition coefficient of a given solvent between 1-octanol and water) values from 1.9 to 4.5, whereas solvents with log P from -1.1 to 0.5 showed the low propagation rate.[162] Among the solvents examined, toluene was the best to produce high molecular weight poly(ϵ-CL) efficiently. Variation in the ratio of toluene to ϵ-CL in the reaction at 70 °C showed that the monomer conversion and polymer molecular weight were the largest with a ratio of about 2:1. Furthermore, lipase CA could be reused for the polymerization. In the range of five cycles, the catalytic activity hardly changed.[148b]

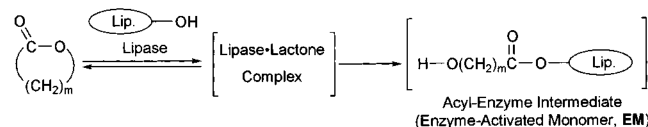
α-Methyl-substituted medium-size lactones, α-methyl-δ-valerolactone (six-membered) and α-methyl-ϵ-caprolactone (seven-membered), were polymerized by lipase CA in bulk to give the corresponding polyesters with M_n of several thousands.[163] Lipase PC induced the enantioselective polymerization of 3-methyl-4-oxa-6-hexanolide (seven-membered); the apparent initial rate of the (S)-isomer was seven times larger than that of the antipode.[164]

Lipase-catalyzed ring-opening polymerization of nine-membered lactone, 8-octanolide (OL), has been reported.[165] Lipases CA and PC showed the high catalytic activity for the polymerization. Racemic fluorinated lactones with a ring size from 10 to 14 were enantioselectively polymerized by lipase CA catalyst to give optically active polyesters.[166]

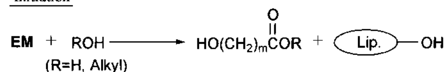
Enzymatic polymerization of macrolides has been extensively studied.[167] So far, four unsubstituted macrolides, 11-undecanolide (12-membered, UDL), 12-dodecanolide (13-membered, DDL), 15-pentadecanolide (16-membered, PDL), and 16-hexadecanolide (17-membered, HDL), were reported to be polymerized by various lipases of different origin. For the polymerization of DDL, the activity order of the catalyst was lipase PC > lipase PF > lipase CC > PPL. High molecular weight polymer with M_n higher than 8×10^4 was synthesized from PDL using lipase CA catalyst in toluene. These macrolides were also polymerized even in an aqueous medium.[168]

Immobilized lipase showing high catalytic activity toward the macrolide polymerization was demonstrated.[169] The immobilization of lipase PF on Celite greatly improved the rate of the DDL polymerization. Catalytic activity was further enhanced by the addition of a sugar or poly(ethylene glycol) (PEG) during immobilization. A surfactant-coated enzyme was used

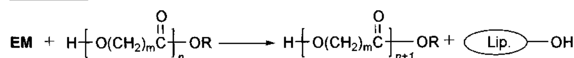
Scheme 22



Initiation



Propagation



as catalyst for the polymerization of PDL.[170] The reaction rate and molecular weight of the resulting polymer using surfactant-coated lipase PC were larger than those by native lipase, suggesting improvement of the catalytic activity by the surfactant coating of lipase.

It is well-known that the catalytic site of lipase is a serine residue and that lipase-catalyzed reactions proceed via an acyl−enzyme intermediate. The enzymatic polymerization of lactones is explained by considering the following reactions as the principal reaction course (Scheme 22).[167a,171] 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 contained partly in the enzyme, onto the acyl carbon of the intermediate to produce ω-hydroxycarboxylic acid ($n = 1$), the shortest propagating species. In the propagation stage, the intermediate is nucleophilically attacked by the terminal hydroxyl group of a propagating polymer to produce a one-unit-more elongated polymer chain. This is an "activated monomer mechanism" in contrast to an "active chain-end mechanism", the widely known polymerization mechanism for vinyl monomers.

In cyclic compounds, reactivity is generally dependent upon their ring size; the ring strain of small and moderate ring size compounds is larger than that of macrocyclic ones, and hence, they show higher ring-opening reactivity. Table 2 summarizes dipole moment values and reactivities of lactones with different ring size. The dipole moment values of the monomers can be taken as an indication of their ring strain. The values of the macrolides are lower than that of ϵ-CL and close to that of an acyclic fatty acid ester (butyl caproate). 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.

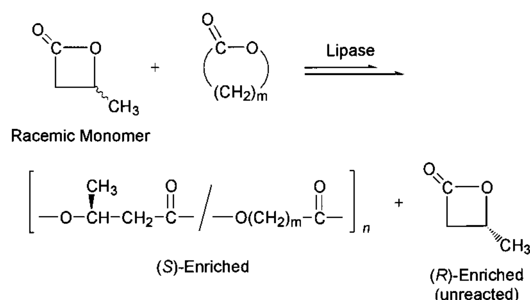
On the other hand, the macrolides showed unusual reactivity toward enzymatic catalysis. Lipase PF-catalyzed polymerization of the macrolides proceeded much faster than that of ϵ-CL. In the evaluation of the enzymatic polymerizability of lactones by Michaelis−Menten kinetics, linearity in the Hanes−Woolf plot was observed for all monomers, indicating that the polymerization followed Michaelis−Menten kinetics.[172] The $V_{max(lactone)}$ and $V_{max(lactone)}/K_{m(lactone)}$ val-

Table 2. Dipole Moments and Reactivities of Unsubstituted Lactones

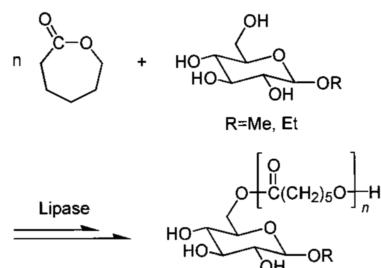
lactone	dipole moment (Cm)	rate constant: alkaline hydrolysis[a] (x10^4, L·mol^{-1}·s^{-1})	rate constant: propagation[b] (10^3, s^{-1})	Michaelis–Menten kinetics[c]: $K_{m(lactone)}$ (mol·L^{-1})	$V_{max(lactone)}$ (x10^2, mol·L^{-1}·h^{-1})	$V_{max(lactone)}/K_{m(lactone)}$ (x10^2, h^{-1})
δ-VL	4.22	55 000				
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 caproate	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 mol·L^{-1}) in diisopropyl ether (10 mL) at 60 °C.

Scheme 23



Scheme 24



ues increased with the ring size of lactone, whereas the $K_{m(lactone)}$ values scarcely changed. These data imply that the enzymatic polymerizability increased as a function of the ring size, and the large enzymatic polymerizability is governed mainly by the reaction rate (V_{max}) but not to the binding abilities, i.e., the reaction process of the lipase–lactone complex to the acyl–enzyme intermediate is the key step of the polymerization.[171]

The kinetics of the ε-CL bulk polymerization by lipase CA showed linear relationships between the monomer conversion and M_n of the polymer; however, the total number of polymer chains was not constant during the polymerization.[173] The monomer consumption apparently followed a first-order rate law.

The lipase-catalyzed copolymerization of lactones often afforded random copolyesters, despite the different enzymatic polymerizability of lactones in some cases.[165,167g,174] So far, the random copolymers were enzymatically obtained from combinations of δ-VL-ε-CL, ε-CL-OL, ε-CL-PDL, and OL-DDL. The formation of the random copolymers suggests that the intermolecular transesterifications of the polyesters frequently took place during the copolymerization. By utilizing this specific lipase catalysis, random ester copolymers were synthesized by the lipase-catalyzed polymerization of macrolides in the presence of poly(ε-CL).[175]

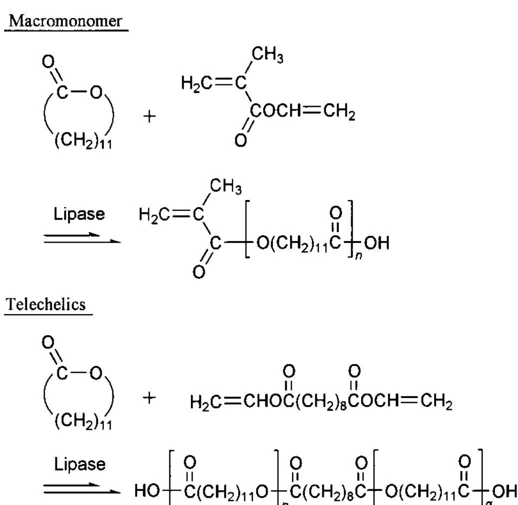
In the lipase-catalyzed polymerization of racemic β-BL, the enantioselectivity was low. The enantioselectivity greatly improved by the copolymerization with ε-CL or DDL using lipase CA catalyst, yielding the optically active polyester with ee up to 69% (Scheme 23).[176] It is to be noted that in the case of lipase CA catalyst, the (*S*)-isomer was preferentially reacted to give the (*S*)-enriched optically active copolymer. The lipase CA-catalyzed enantioselective copolymerization of δ-caprolactone (six-membered) with DDL produced the optically active polyester with ee of 76%.

Lipase catalysis provided new methodologies for single-step functionalization of polymer terminal. As shown in Scheme 22, an alcohol acted as an initiating species in the ring-opening polymerization of lactones by lipase CA catalyst to introduce the alcohol moiety at the polymer terminal ("initiator method").[177] In the polymerization of DDL employing 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. This methodology was expanded to synthesis of ω-alkenyl- and alkynyl-type macromonomers by using 5-hexen-1-ol and 5-hexyn-1-ol as initiator.

Polyesters bearing a sugar moiety at the polymer terminal were synthesized by lipase CA-catalyzed polymerization of ε-CL in the presence of alkyl glucopyranosides (Scheme 24).[178] In the initiation step, the regioselective acylation at the 6-position of the glucopyranoside took place. Polysaccharides also initiated the lipase-catalyzed polymerization of ε-CL.[179] The enzymatic graft polymerization of ε-CL on hydroxyethyl cellulose produced cellulose-*graft*-poly(ε-CL) with the degree of substitution from 0.10 to 0.32. A poly(ε-CL)-monosubstituted first-generation dendrimer was synthesized by using lipase CA, in which the initiator was selectively monoacylated at the initial stage.[180]

Polyester macromonomers and telechelics were synthesized by the lipase-catalyzed polymerization of DDL using vinyl esters as terminator ("terminator method").[181] In using vinyl methacrylate and lipase PF as terminator and catalyst, respectively, the quantitative introduction of methacryloyl group at the polymer terminal was achieved to give the

Scheme 25



methacryl-type macromonomer (Scheme 25). By addition of divinyl sebacate, the telechelic polyester having a carboxylic acid group at both ends was obtained.

Lipase-catalyzed ring-opening polymerization of cyclic diesters was investigated. Lactide was polymerized using lipase PC as catalyst at high temperatures (80−130 °C) to produce poly(lactic acid) with M_w higher than 1×10^4.[182] D,L-Lactide enzymatically gave a polymer with a higher molecular weight than the D,D- and L,L-isomers.

Lipases CA, PC, and PF catalyzed the polymerization of ethylene dodecanoate and ethylene tridecanoate to give the corresponding polyesters.[183] The polymerization behaviors depended on the lipase origin; in using lipase PC catalyst, these bislactones polymerized faster than ε-CL and DDL, whereas the reactivity of these cyclic diesters was in the middle of ε-CL and DDL in using lipase CA.

Lipase catalyzed the ring-opening polymerization of cyclic carbonates. Trimethylene carbonate (six-membered, TMC) was polymerized by lipases CA, PC, PF, and PPL to produce the corresponding polycarbonate without involving elimination of carbon dioxide. High molecular weight poly(TMC) ($M_w > 1 \times 10^5$) was obtained by using a small amount of PPL catalyst (0.1 or 0.25 wt % for TMC) at 100 °C.[184] Lipase CA induced the polymerization under milder reaction conditions.[185] The sugar-terminal poly(TMC) was synthesized by the lipase CA-catalyzed polymerization in the presence of methyl glucopyranoside.[178a]

Chemoenzymatic synthesis of a water-soluble polycarbonate having pendent carboxyl groups on the polymer main chain was achieved by lipase-catalyzed polymerization of 5-methyl-5-benzyloxycarbonyl-1,3-dioxan-2-one (MBC), followed by debenzylation.[186] The copolymerization of MBC with TMC using lipase PF catalyst produced the random copolycarbonate.[187]

Cyclic dicarbonates, cyclobis(hexamethylene carbonate) and cyclobis(diethylene glycol carbonate), were polymerized by lipase CA.[188] The random ester−carbonate copolymers were enzymatically obtained from DDL-cyclobis(diethylene glycol carbonate) and lactides-TMC.[189]

Besides cyclic esters and carbonates, six-membered cyclic depsipeptides and a five-membered cyclic phosphate were subjected to lipase-catalyzed ring-opening polymerizations, yielding poly(ester amide)s[190] and polyphosphate,[191] respectively. High temperatures (100−130 °C) were required for the polymerization of the former monomers.

2. Polymerization of Diacid Derivatives and Glycols

So far, biotransformations of various combinations of dicarboxylic acid derivatives and glycols to biodegradable polyesters have been reported. Dicarboxylic acids as well as its derivatives, activated and nonactivated esters, cyclic acid anhydride, and polyanhydrides, were found to be employed as useful monomers for the enzymatic synthesis of polyesters under mild reaction conditions.

Many dicarboxylic acids and their alkyl esters are commercially available; however, they often showed low reactivity toward lipase catalyst. Thus, development of the reaction apparatus and reaction conditions has been made for efficient production of higher molecular weight polyesters. In the polycondensation of adipic acid and 1,4-butanediol, a horizontal two-chamber reactor was employed to remove the leaving water molecules with use of molecular sieves.[192] A low-dispersity polyester with a degree of polymerization (DP) of ~20 was obtained by the two-stage polymerization using *Mucor miehei* lipase (lipase MM).

Enzymatic synthesis of aliphatic polyesters from diacids and glycols in a solvent-free system was carried out.[193] Lipase CA catalyzed the polymerization under mild reaction conditions to give the polymer with M_n of several thousands, despite the heterogeneous mixture of the monomers and catalyst. The polymerization behaviors strongly depended on the chain length of both monomers. 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 a more than 200 kg yield. This solvent-free system claimed a large potential as 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 nonaqueous media. Since a product water of the dehydration is in equilibrium with starting materials, a solvent water disfavors the dehydration to proceed in an aqueous medium due to the “law of mass action”. However, dehydration polycondensation of dicarboxylic acids and glycols proceeded even in water by using lipase catalyst.[194] Various lipases such as lipases CA, CC, and MM catalyzed the polymerization of sebacic acid and 1,8-octanediol. In the polymerization of α,ω-dicarboxylic acids and glycols, the chain length of the monomers strongly affected the polymer yield and molecular weight; the polymers from 1,12-docecanedioic acid and 1,10-decanediol were obtained in good yields, whereas no polymer formation was observed in using 1,6-hexanediol.

The polymerization using dialkyl esters also produced the polyesters with relatively low molecular

weight. Lipase CA- or MM-catalyzed polycondensation of dimethyl succinate and 1,6-hexanediol in toluene quickly attained the equilibrium (the conversion of methyl ester moiety was ca. 50%); however, M_n of the product was very low (ca. 300).[195] The equilibrium was shifted by elimination of methanol with nitrogen bubbling, leading to an increase of conversion (>99%) and of the polymer molecular weight ($M_n \approx 3 \times 10^3$). Formation of cyclic oligomers with DP from 2 to 20 as byproduct was observed, and their yield depended on the monomer structure and concentration and reaction temperature. The ring-chain equilibrium was observed, and the molar distribution of the cyclic species obeys the Jacobson–Stockmayer equation.

The molecular weight greatly increased when a vacuum was used in the polymerization using diacids or dialkyl esters as monomer; removal of the formed water or alcohols resulted in a shift of the equilibrium toward the product polymer. In the lipase MM-catalyzed polymerization of sebacic acid or its ethyl ester with 1,4-butanediol in hydrophobic solvents of high boiling points such as diphenyl ether and veratrole under vacuum, M_w of the product polymer reached higher than 4×10^4, although a long reaction time (>1 week) was required.[196]

In lipase-catalyzed esterifications and transesterifications, esters of halogenated alcohols, typically 2-chloroethanol, 2,2,2-trifluoroethanol, and 2,2,2-trichloroethanol, have been used often, owing to an increase of the electrophilicity (reactivity) of the acyl carbonyl and avoiding significant alcoholysis of the products by decreasing the nucleophilicity of the leaving alcohols. The enzymatic synthesis of biodegradable polyesters from the activated diesters was achieved under mild reaction conditions.[197] The polymerization of bis(2,2,2-trichloroethyl) glutarate and 1,4-butanediol at room temperature in diethyl ether gave the polyesters with $M_n \approx 8 \times 10^3$. The polymerization of bis(2,2,2-trichloroethyl) adipate and 1,4-butanediol using PPL catalyst proceeded in a supercritical fluoroform solvent to give the polymer with M_w of several thousands.[198] By changing the pressure, the low-dispersity polymer fractions were separated.

Vacuum technique was applied to shift the equilibrium forward by removal of the activated alcohol formed.[196,199] In the enzymatic polycondensation of bis(2,2,2-trifluoroethyl) sebacate and aliphatic diols, lipases CR, MM, PC, and PPL produced the polymer with M_w of more than 1×10^4 and lipase MM showed the highest catalytic activity. In the PPL-catalyzed reaction of bis(2,2,2-trifluoroethyl) glutarate with 1,4-butanediol in veratrole or 1,3-dimethoxybenzene, the periodic vacuum method increased the molecular weight ($M_w \approx 4 \times 10^4$).

Enol esters have been shown to be good acylating reagents in lipase-catalyzed reactions, since the leaving unsaturated alcohol irreversibly tautomerizes to an aldehyde or a ketone, leading to the desired product in high yields (see also Scheme 25). Thus, the enzymatic polymerization using divinyl adipate and 1,4-butanediol was first demonstrated in 1994;[200a] the reaction proceeded in the presence of lipase PF at 45 °C. Under similar reaction conditions, adipic acid and diethyl adipate did not afford the polymeric materials, indicating the high polymerizability of bis(enol ester) toward lipase catalyst. In the enzymatic polymerization of divinyl adipate or divinyl sebacate with α,ω-glycols with different chain lengths, lipases CA, MM, PC, and PF showed high catalytic activity toward the polymerization.[200] A combination of divinyl adipate, 1,4-butanediol, and lipase PC afforded the polymer with $M_n \approx 2 \times 10^4$. 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. The polyester formation was observed in various organic solvents, and among them, diisopropyl ether gave the best results. The polymerization also proceeded in bulk by using lipase CA as catalyst.

In the case of polyester synthesis from divinyl esters, hydrolysis of the vinyl end group partly took place, resulting in the limitation of the polymer growth.[201] A mathematical model showing the kinetics of the polymerization predicts the product composition. On the basis of these data, a batch-stirred reactor was designed to minimize temperature and mass-transfer effects.[202] The efficient enzymatic production of polyesters was achieved using this reactor; poly(1,4-butylene adipate) with $M_n \approx 2 \times 10^4$ was synthesized in 1 h at 60 °C.

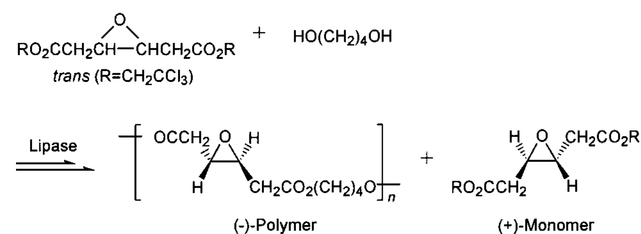
Ester copolymers were synthesized by lipase-catalyzed copolymerization of lactones, divinyl esters, and glycols.[203] Lipases CA and PC showed high catalytic activity for this copolymerization, yielding ester copolymers with M_n higher than 1×10^4. ^{13}C NMR analysis showed that the resulting product 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.

Another approach based on the irreversible process was performed by using bis(2,3-butanedione monoxime) alkanedioates as diester substrate.[204] Lipase MM efficiently produced the polymer with M_n up to 7.0×10^3.

Cyclic anhydrides and polyanhydrides were used as starting substrate for enzymatic production of polyesters. A new type of enzymatic polymerization involving lipase-catalyzed ring-opening poly(addition–condensation) of cyclic acid anhydride with glycols was demonstrated.[205] The polymerization of succinic anhydride with 1,8-octanediol using lipase PF catalyst proceeded at room temperature to give the polyester. The enzymatic reaction of poly(azelaic anhydride) and glycols using lipase CA catalyst also produced the polyesters in which glycols are apparently inserted into poly(azelaic anhydride).[206]

Polymerization of oxiranes with succinic anhydride proceeded in the presence of PPL catalyst.[207] Under appropriate conditions, M_w reached 1×10^4. During the polymerization, the enzymatically formed acid group from the anhydride may open the oxirane ring to give a glycol, which is then reacted with the anhydride or acid by lipase catalysis, yielding polyesters.

Scheme 26



Aromatic polyesters were enzymatically synthesized under mild reaction conditions. Divinyl esters of isophthalic acid, terephthalic acid, and *p*-phenylene diacetic acid were polymerized with glycols by lipase CA catalyst to give polyesters containing an aromatic moiety in the main chain.[208] In the lipase-catalyzed polymerization of dimethyl isophthalate and 1,6-hexanediol in toluene with nitrogen bubbling, a mixture of linear and cyclic polymers was formed.[209] High molecular weight aromatic polyester ($M_w \approx 5.5 \times 10^4$) was synthesized by the lipase CA-catalyzed polymerization of isophthalic acid and 1,6-hexanediol under vacuum.[210] Enzymatic polymerization of divinyl esters and aromatic diols also afforded the aromatic polyesters.[211]

Functional polyesters have been synthesized by utilizing characteristic catalysis of lipase. An optically pure polyester was synthesized by PPL-catalyzed enantioselective polymerization of bis(2,2,2-trichloroethyl) *trans*-3,4-epoxyadipate with 1,4-butanediol in diethyl ether (Scheme 26).[212] The molar ratio of the diester to the diol was adjusted to 2:1 to produce the (−)-polymer with an enantiomeric purity of >96%. From end group analysis, the molecular weight was calculated to be 5.3×10^3.

Lipase-catalyzed regioselective polymerization of divinyl esters and polyols to the soluble polymers with relatively high molecular weight was achieved. In the lipase CA-catalyzed polymerization of divinyl sebacate and glycerol at 60 °C, 1,3-diglyceride was the main unit of the formed polymer and a small amount of the branching unit (triglyceride) was contained.[213] The regioselectivity of the acylation between primary and secondary hydroxy groups was 74:26. Under the selected reaction conditions, the regioselectivity was perfectly controlled to give a linear polymer consisting exclusively of a 1,3-glyceride unit. The lipase CA-catalyzed polymerization of divinyl esters and sorbitol regioselectively proceeded to produce the sugar-containing polyester with the 1,6-diacylated unit of sorbitol.[214] Mannitol and *meso*-erythritol were also regioselectively polymerized with divinyl sebacate.

Two-step enzymatic synthesis of polyesters containing a sugar moiety in the main chain was demonstrated.[215] Sugar diesters were synthesized by lipase CA-catalyzed esterification of sucrose or trehalose with divinyl adipate in acetone, in which the 6- and 6′-positions of the starting sugar were regioselectively acylated. The same enzyme catalyzed the subsequent polycondensation of the isolated diesters with glycols to give the sugar-containing polyesters with M_w up to 2.2×10^4.

A new class of cross-linkable polyesters having an unsaturated group in the side chain was synthesized by the lipase CA-catalyzed polymerization of divinyl sebacate and glycerol in the presence of unsaturated higher fatty acids.[216] The polyester was subjected to hardening by cobalt naphthenate catalyst or thermal treatment, yielding cross-linked transparent film.

All-trans unsaturated ester oligomers have been synthesized by lipase-catalyzed polymerization of diesters of fumaric acid and 1,4-butanediol.[217] No isomerization of the double bond was observed, as opposed to the extensive isomerization found during chemical polycondensations. Crystallinity was found in the enzymatically formed unsaturated oligoesters prepared in acetonitrile, whereas industrial unsaturated polyesters are amorphous.

The lipase CA-catalyzed polymerization of dimethyl maleate and 1,6-hexanediol proceeded using lipase CA catalyst in toluene to produce a mixture of linear and cyclic polymers exhibiting exclusively cis structure.[218] The cyclics were semicrystalline, whereas the linear polymer was amorphous. In the copolymerization of dimethyl maleate and dimethyl fumarate with 1,6-hexanediol by lipase CA catalyst, the content of the cyclization was found to mainly depend on the configuration and concentration of the monomers.[219]

Chemoenzymatic synthesis of alkyds (oil-based polyester resins) was reported.[220] PPL-catalyzed transesterification of triglycerides with an excess of 1,4-cyclohexanedimethanol mainly produced 2-monoglycerides, followed by thermal polymerization with phthalic anhydride to give the alkyd resins with a molecular weight of several thousands. Reaction of the enzymatically obtained alcoholysis product with toluene diisocyanate produced the alkyd−urethanes.

Fluorinated polyesters were synthesized by the enzymatic polymerization of divinyl adipate with fluorinated diols.[221] Using 3,3,4,4,5,5,6,6-octafluorooctan-1,8-diol as glycol monomer in the lipase CA-catalyzed polymerization in bulk produced the polymer with the highest molecular weight ($M_n \approx 5 \times 10^3$).

Polycarbonate synthesis by lipase-catalyzed polycondensation was demonstrated. Activated dicarbonate, 1,3-propanediol divinyl dicarbonate, was used as the monomer for enzymatic synthesis of polycarbonates.[222] Lipase CA-catalyzed polymerization with α,ω-alkylene glycols produced the polycarbonates with M_w up to 8.5×10^3. Aromatic polycarbonates with DP larger than 20 were enzymatically obtained from the activated dicarbonate and xylylene glycols in bulk.[211]

Higher molecular weight polycarbonate was enzymatically synthesized from diethyl carbonate.[223] The lipase CA-catalyzed bulk reaction of an excess of diethyl carbonate with 1,3-propanediol or 1,4-butanediol under ambient pressure gave oligomeric products followed by polymerization under vacuum to give aliphatic polycarbonates with M_w higher than 4×10^4.

3. Polycondensation of Oxyacid Derivatives

Enzymatic polymerizations of oxyacid derivatives have been reported; however, in most cases, only low molecular weight polyesters (molecular weight less or about 1×10^3) were formed.[147] In the PPL-catalyzed polymerization of 12-hydroxydodecanoic

acid at 75 °C for 56 h, M_n reached 2.9×10^3.[224] Loading a large amount of lipase increased the molecular weight.[225] Use of 10 weight fold lipase CR for 10-hydroxydecanoic or 11-hydroxyundecanoic acid afforded the polyester of relatively high molecular weight (M_w up to 2.2×10^4) in the presence of activated molecular sieves.

PPL catalyzed the polymerization of methyl esters of 5-hydroxypentanoic and 6-hydroxyhexanoic acids.[149] In the polymerization of the latter in hexane at 69 °C for more than 50 days, the polymer with DP up to 100 was formed. Relationships between solvent type and polymerization behaviors were systematically investigated; hydrophobic solvents such as hydrocarbons and diisopropyl ether were suitable for the enzymatic production of high molecular weight polymer. *Pseudomonas* sp. lipase catalyzed the polymerization of ethyl esters of 3- and 4-hydroxybutyric acids, 5- and 6-hydroxyhexanoic acids, 5-hydroxydodecanoic acid, and 15-hydroxypentadecanoic acid.[157] Oxyacid vinyl esters were demonstrated as new monomers for polyester production under mild reaction conditions, yielding the corresponding polyesters with M_n of several thousands.[27b]

Enzymatic regioselective polymerization of cholic acid was reported. A hydroxy group at the 3-position was regioselectively acylated by lipase CA catalyst to give the oligoester with molecular weight less than 1×10^3.[226]

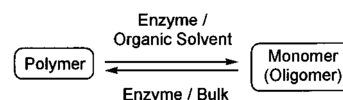
Optically active polyesters were enzymatically obtained from oxyacid derivatives. In the lipase CR-catalyzed polymerization of racemic 10-hydroxyundecanoic acid, the resulting oligomer was enriched in the (*S*)-enantiomer to a level of 60% ee and the residual monomer was recovered with a 33% ee favoring the antipode.[227] Lipase CA catalyzed the polymerization of lactic acid to give the corresponding oligomer with DP up to 9, in which the *R*-enantiomer possessed higher enzymatic reactivity.[228] Optically active oligomers (DP < 6) were also synthesized from racemic ϵ-substituted-ϵ-hydroxy esters using PPL catalyst.[229] The enantioselectivity increased as a function of bulkiness of the monomer substituent. The enzymatic copolymerization of the racemic oxyacid esters with methyl 6-hydroxyhexanoate produced the optically active polyesters with a molecular weight higher than 1×10^3.

In the polymerization of 12-hydroxydodecanoic acid in the presence of 11-methacryloylaminoundecanoic acid using lipases CA or CR as catalyst, the methacrylamide group was quantitatively introduced at the polymer terminal, yielding a polyester macromonomer.[230]

4. Polymer Modification

Lipase catalysts have been used for functionalization of polymers. A terminal hydroxy group of poly(ϵ-CL) was reacted with carboxylic acids using lipase CA catalyst to give end-functionalized polyesters.[231] Lipase MM catalyzed the regioselective transesterification of the terminal ester group of oligo(methyl methacrylate) with allyl alcohol.[232] In the PPL-catalyzed reaction of racemic 2,2,2-trichloroethyl 3,4-epoxybutanoate with hydroxy-terminated PEG, the

Scheme 27



(*S*)-isomer of the ester was enantioselectively introduced at the PEG terminal (ee ≥ 89%).[233]

Ester copolymers were synthesized by the lipase-catalyzed intermolecular transesterification between two different polyesters, poly(ϵ-CL) and poly(PDL).[175,234] Under selected conditions, lipase could act as a hydrolytic degradation catalyst of polyesters.[235] A low concentration of poly(ϵ-CL) with a molecular weight $\approx 4 \times 10^4$ in toluene was readily subjected to the degradation in the presence of lipase CA catalyst to give oligomers with molecular weights less than 500. The degradation behavior catalyzed by lipase was quite different than an acid-catalyzed degradation of 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 27). Cyclic dicaprolactone was selectively formed from a very dilute solution of poly(ϵ-CL).

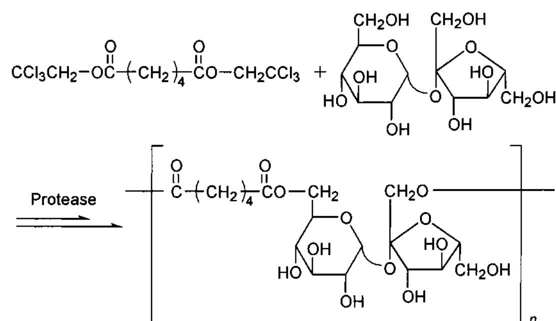
Lipase is known as a catalyst for epoxidation with peroxides. Polybutadiene was subjected to lipase CA-catalyzed oxidation of polybutadiene using hydrogen peroxide as the oxidizing agent in the presence of acetic acid under mild reaction conditions.[236] The epoxide rings formed were opened and esterified.

C. Proteases

As seen for the hydrolysis enzyme, proteases catalyze not only hydrolysis of peptide bonds but also peptide bond formation. The reaction of amino acid esters in the presence of some proteases produces water-insoluble products. Ester hydrochlorides of methionine, phenylalanine, threonine, and tyrosine were polymerized by papain catalyst to give poly(amino acid)s with DP less than 10.[237] In using racemic substrates, only L-isomers were stereoselectively polymerized. Papain and α-chymotrypsin induced the polymerization of diethyl L-glutamate hydrochloride to give the polymer consisting exclusively of α-peptide linkage.[238] Diethyl L-aspartate was polymerized by alkanophilic protease from *Streptomyces* sp. to give the polymer with a mixed structure of α- and β-peptide linkages.[239]

Protease mutants were prepared, which showed higher catalytic activity for the enzymatic polymerization of amino acid esters in an aqueous DMF solution. The molecular weight greatly increased by using a subtilisin mutant (subtilisin 8350) derived from BPN′ (subtilisin from *Bacillus amyloliquefaciens*) via six site-specific mutants (Met 50 Phe, Gly 169 Ala, Asn 76 Asp, Gln 206 Cys, Tyr 217 Lys, and Asp 218 Ser) in the polymerization of L-methionine methyl ester in the aqueous DMF.[240] Another mutant (subtilisin 8397), which is the same as 8350 without

Scheme 28



changing Tyr 217, induced the polymerization of single amino acid, dipeptide, and tripeptide methyl esters.[241]

A different type of peptide hydrolase, dipeptide transferase, catalyzed the oligomerization of dipeptide amides. In the case of glycyl-L-tyrosinamide, the corresponding oligomer with DP up to 8 was formed.[242]

Some proteases show an esterase activity, especially in their catalytic activity for regioselective acylation of sugars. Protease-catalyzed synthesis of polyester containing a sugar group in the backbone has been achieved.[243] Polycondensation of sucrose with bis(2,2,2-trifluoroethyl)adipate using an alkaline protease from *Bacillus* sp. as catalyst proceeded regioselectively to give an oligoester having ester linkages at the 6- and 1′-positions on the sucrose (Scheme 28). The regioselective catalysis of protease was applied for the modification of polysaccharides.[244] Protease (subtilisin Carlsberg) solubilized in isooctane catalyzed the acylation of amylose film with vinyl caprate only at the 6-position of amylose.

D. Other Hydrolases

PHB depolymerases showed catalytic activity for the ring-opening polymerization of cyclic monomers. PHB depolymerase from *Pseudomonas stutzeri* YM1006 polymerized β-BL, and the catalytic activity of the enzyme without substrate-binding domains (SBD) was larger than that with SBD.[245] ϵ-CL and TMC were polymerized by PHB depolymerase from *Pseudomonas lemoignei* to give the oligomers with DP of 4 and 12, respectively.[246]

Epoxide hydrolases, which are contained in crude lipases, induced the ring-opening oligomerization of glycidol in bulk to give a water-soluble oligomer with $M_w \approx 1 \times 10^3$.[247] The resulting oligomer was of a branched structure.

VI. Conclusion

The present review describes recent developments on in vitro polymer production using an isolated enzyme as catalyst via nonbiosynthetic pathways (enzymatic polymerization). Beyond the in vivo relationship of the key-and-lock theory, in vitro catalysis of enzymes allowed structural variation of monomers and polymers, leading to not only natural polymers but unnatural polymers including new useful materials. In many cases, enzymes catalyzed highly enantio-, regio-, and chemoselective as well as stereoregulating polymerizations to produce a variety of functional polymers and structurally complicated polymers, syntheses of which are very difficult to be achieved via conventional chemical routes. Such typical examples may be artificial cellulose, chitin, hyaluronic acid, urushi, etc.

Recently, genetic engineering has been significantly developed to produce tailor-made mutant enzymes (artificial enzymes). Accumulated knowledge of research on catalytic antibodies is also informative for developing new enzyme catalysts.[3b] Thus, these biocatalysts showing high catalytic activity, reaction selectivity, or stability in organic solvents can eventually be designed and prepared on the basis of relationships between the structure and function of enzymes. These developments will broaden the scope of precision enzymatic syntheses of numerous kinds of polymers. In addition, the enzymatic processes for production of useful polymeric materials are environmentally highly benign, since in most cases biodegradable products are obtained from nontoxic substrates and catalysts under mild reaction conditions. Therefore, in the future, enzymatic polymerizations are expected to provide essential technology in chemical industry.

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