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REVIEW

POLYMERS FROM BIOCATALYSTS

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Abstract – Recently, there has been great interest in enzyme-catalyzed polymer synthesis, particularly as an alternative to conventional polymer chemistry. Highly specific and stable biocatalysts can result in increased yields and reduced capital requirements and production costs. Moreover, the use of biocatalysts can minimize the formation of unwanted byproducts and thus reduce separation cost. Enzymatic synthesis of polymer is especially preferred when molecular regularity such as stereoselectivity or regiospecificity is required. In this article, the use of enzymes in polymer synthesis is discussed together with potential applications.

Key words : Enzymatic Polymerization, Biocatalyst, Polymer, Polymerization, Chemoenzymatic Synthesis

INTRODUCTION

The use of enzymes or whole cells as catalysts in specialty chemical synthesis during the last ten years has become a useful technique [Hairston, 1995; Williams et al., 1996; Kobayashi et al., 1992]. The hallmark of biocatalysts is their high regio- and enantioselectivities [Santhanam and Shreve, 1994; Wescott and Klibanov, 1994; Francalanci et al., 1987], and this has been a major driving force for using them as catalysts in polymer synthesis [Turner, 1995; Laval et al., 1995; Asano et al., 1982].

There is a great opportunity for using biotechnology to provide a novel synthetic route to useful polymeric materials from non-petrochemical renewable resources. Biologically generated polymers possess peculiar properties such as biodegradability and biocompatibility. New trends in polymer synthesis are derived from researches involving *in vitro* specialty chemical synthesis using biocatalyst [Dordick, 1996; Uyama et al., 1995; Wang et al., 1995d; Lalot et al., 1996]. Currently, several types of polymers are commercialized using biological means: polysaccharides, polyesters, polypeptides, polyphenols, nucleic acids, fibers, etc. Typical examples are the cellulose synthesis by cellulase [Kobayashi, 1996], and phenolic polymerization using peroxidase enzyme [Enzymol International Inc.; Dordick et al., 1986; Dordick et al., 1987].

In particular, enzymatic polymerization in organic solvents is of increasing importance, and in recent years a rapid expansion in this area has taken place. Two decades ago, a work by Klibanov [Klibanov et al., 1977] initiated the enzyme-catalyzed polymerization reactions in an anhydrous organic solvent media. Enzymatic polymerization in organic solvent may be of interest with respect to the increase of polymer solubility, favorable shifting of thermodynamic equilibrium (from hydrolysis to polymerization), and enzymatic resolution of polymer enantio-/regioselectivity, etc. [Klibanov, 1986; Wallace and Morrow, 1989a; Wang et al., 1995d; Lilly and Woodley, 1985; Riva et al., 1988]. The technology, nowadays, has led to one of the most advanced researches in the field of biocatalyst engineering as well as enzyme-catalyzed specialty polymer synthesis. For example, in organic solvent media, a sugar-based linear poly (sugar acrylate) has been produced that has a molecular weight (Mn) as high as 2×10^6 [Chen et al., 1995]. Recently, researchers at Helsinki University of Technology (Espoo, Finland) achieved a world record [unpublished data, 1996]; biodegradable polymer with nearly 77,000 weight average molar mass (g mol^{-1}) was synthesized through lipase-catalyzed polyesterification. Poly (1,4-butyl sebacate) was synthesized from sebacic acid and 1, 4-butanediol. Another example is the enzymatic synthesis of artificial sweetener L-a-aspartame (L-Asp-L-Phe-OMe; APM) in organic solvent media carried out by researchers at Toyo Soda Manufacturing Co, Ltd. (Yamaguchi, Japan) [Oyama, 1986]. Both stereoselectivity and an equilibrium shift were achieved in ethyl acetate-water (7 : 3 in weight ratio).

Current trends in the preparation of chiral polymers, the preparation of lignin-like compounds such as poly(phenol)s, chemoenzymatic and multi-enzymatic polymer synthesis, diversification of enzymatic polymer synthesis, and new strategy for the enzyme-derived polymerization will be discussed.

DIRECT SYNTHESIS OF POLYMERS

1. Polyester Synthesis

The possibility of preparing polyesters having high molecular weights (Mw : 5,000-15,000 g mol^{-1}) by enzyme-catalyzed transesterification in organic solvent media was investigated (Fig. 1A) [Wallace and Morrow, 1989]. Most of these transformations resulted in high regio- and/or stereoselectivity. While these esterification reactions can be performed chemically, optically active monomers have to be employed to give optical selectivity in the case of chemical synthesis. Previous attempts

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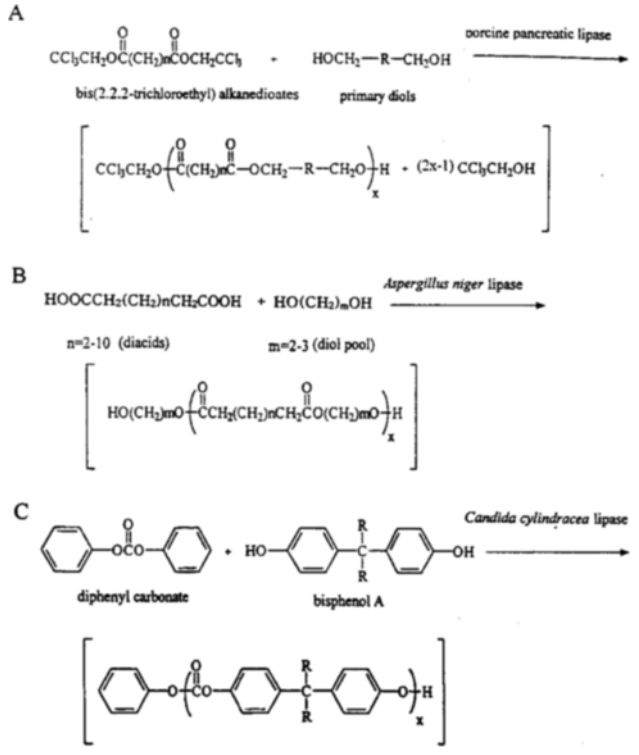

Fig. 1. Lipase-catalyzed polymerizations of linear polyesters [Wallace and Morrow, 1989; Okumura et al., 1984; Abramowicz and Keese, 1989].

for enzymatic polyesterification were demonstrated by several researchers [Okumura et al., 1984; Ajima et al., 1985]. Okumura and his coworkers reported the *Aspergillus niger* lipase (ANL)-catalyzed oligomerization of 1,2-ethanediol and 1,3-propandiol with the diacids from 1,6-hexandioic acid through 1,14-tetradecanedioic acid (Fig. 1B). The products proved to be 2-3 mers of the form $[AA\text{-}BB]_n$, which were formed from 1,3-propandiol (AA), and 1,3-tridecanedioic acid (BB), after 24 hours of the reaction. The A-B type polymer was first synthesized by Ajima and his co-workers [Ajima et al., 1985]. In this case, 10-decanoic was used as a monomer. Another approach for preparing a polycarbonate by an enzyme-catalyzed process was reported in 1989 [Abramowicz and Keese, 1989]. The transesterification of diphenyl carbonate with bisphenol A, as well as with a range of simple alcohols in water-saturated ether solvent, resulted in oligomers having molecular weights of up to 900 g mol^{-1}. The enzymes used for the study were porcine liver esterase and lipase from yeast Candida cylindracea (Fig. 1C).

Polyhydroxybutyrate (PHB) was the first discovered polyester from whole cell systems (*Alcaligenes species*). Although some useful properties of PHB such as biodegradability and biocompatibility have been carved in relief for more than 20 years, the industrial production of PHB did not occur until 1982, when ICI (UK) marketed them under the commercial name of "*Biopol*" (PHB/V) [ICI PLC, European Patent, 1982]. Aliphatic polyester from Union Carbide (Tone Polymer) has similar structure [Yokota et al., 1994]. Another production system of polyester is using hydrolytic enzymes such as lipases, proteases, etc. These kinds of enzymes are cost effective and now widely used in commercial detergent powders.

In the case of polyesterification, many industrial applications exist. The potential usage of succinic acid-based polyester for synthetic rubber was already investigated in 1947 [Kaminsky, 1992]. Among esterification reactions, lipase catalyzed transesterification is often preferred to direct esterification because no water is formed in that reaction [Wallace and Morrow, 1989a,b; Morrow and Wallace, 1990]. Enzymatic polytransesterification of aromatic diols was carried out in anhydrous organic solvent [Park et al., 1995]. The protease from *Bacillus lichemiformis* catalyzed the polytransesterification of a diester of glutanic acid with aromatic diols such as benzenedimethanol. Gel permeation chromatography provided molecular weight of 400-1,400 daltons for the aromatic polyesters. For the transesterification, monomer selection is also an important factor since the yield and molecular weight can be altered by this factor. Large size monomers do not always result in high molecular weight polymers in the polyesterification reaction. For diol-diacid catalysis using lipase from *Pseudonomas sp.* or *Mucor miehei*, 1,4-butanediol is a widely selected monomer as a diol monomer [Linko et al., 1993; Wang et al., 1995d]. However, there is an engineering problem that must be handled optimally to improve reaction performances (e.g., catalyst selection, control of molecular weight and distribution, etc.). Fig. 2 shows the reaction characteristics of enzyme-catalyzed polyesterification. According to the carbon number of a monomer, the yield and number molecular weight of the synthesized polymer meet a certain maximum value. At a critical monomer size, polyesterification shows good yield and the polymers synthesized from this monomer have a narrow range of molecular weight distribution (Mw/Mn <1.2) [Morrow and Wallace, 1990]. For most cases of lipase catalysis, the critical carbon number of diol monomer is about 4 to 5 [Wallace and Morrow, 1989]. Poor reaction and low molecular weight polymers are shown above

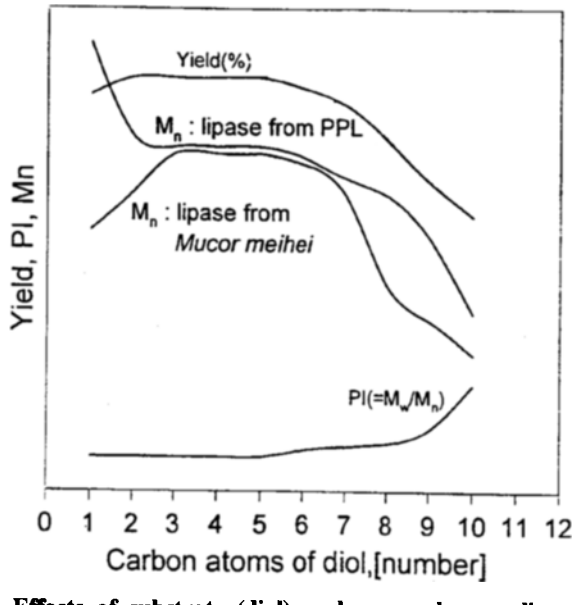

Fig. 2. Effects of substrate (diol) carbon number on lipase-catalyzed polymerization. Substrate: 1,4-butanediol.

the critical carbon number. The difference between ideal catalysis and poor reaction prognosis is related to the catalyst reactivity with the selected monomer. This means that a trade off between the selection of monomer and the catalyst exists with respect to the overall reaction performance and polymer properties which may be targeted.

An enzymatic polymerization including ring-opening of a monomer using a lipase has been reported [Uyama et al., 1995; Gross et al., 1996]. One is a ring-opening homo- or copolymerization of lactones and another is a poly addition-condensation between a cyclic acid anhydride and a glycol. The reactivities of these cyclic compounds generally depend on the size of the ring and the dielectric constant of the monomers. The synthesized polymers showed higher molecular weight (max. Mw : 25,000) than that obtained by conventional anionic polymerization, with a narrow molecular weight distribution. Recently, similar research was carried out [Hendersen et al., 1996; McDonald et al., 1995]. The researchers reported that porcine pancreatic lipase catalyzed ring-opening polymerization of ε-caprolactone using butanol as an initiator (Fig. 3). Also, in their approach, an additional butylamine initiation supports the chain polymerization mechanism with fast initiation and slow propagation.

2. Fatty Acid Polymer Synthesis

Enzymes can polymerize polyol-fatty acid esters [Hayes and Gulari, 1995]. These polymeric (oligomeric having 6-7 mers) fatty acids or their derivatives are useful in edible oils

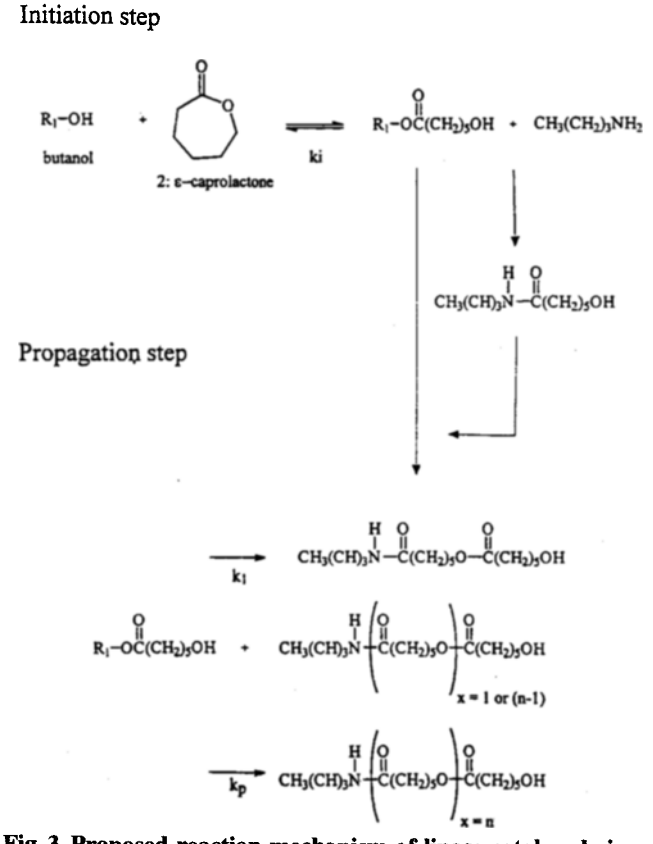

Fig. 3. Proposed reaction mechanism of lipase-catalyzed ring-opening polymerization of ε-caprolactone [Hendersen et al., 1996].

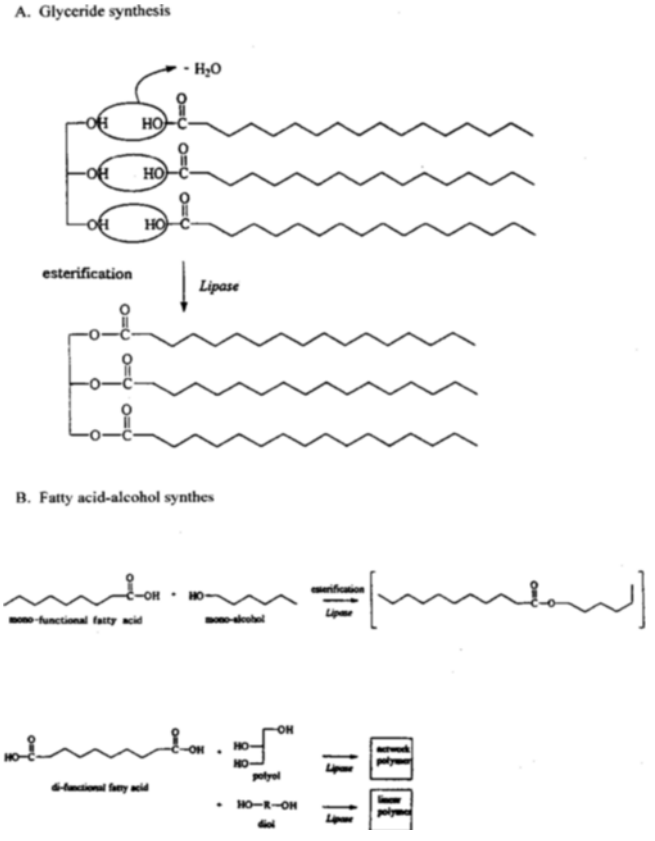

Fig. 4. Examples of fatty acid-alcohol synthesis.

(e.g., cooking oils), high performance lubricants, flavors for cosmetics, and as emulsifiers in printing inks. Typical examples of polyol-fatty acid syntheses are shown in Fig. 4. The degree of polymerization varies significantly in the function of the hydroxy fatty acid type. Modification of these polymers synthesized by lipase is also preferred in many cases for the reasons mentioned above. As a new substitute for edible oils, low-calorie triglycerides that are difficult to perform by conventional means, were synthesized using lipase from *Geotrichum candidum* (or *Candida rugosa*) by a research group in US Dairy Association. It is important to synthesize non-adsorbing fats and edible oils for human diet with respect to human physiology. These enzymatically synthesized triglycerides are almost physicochemically identical to natural fats but rarely absorbed during human digestion. A series of researches on the lipase catalyzed polyol-fatty acids synthesis using reversed micellar media were performed [Hayes, 1996; Hayes and Gulari, 1992]. They reported an interesting result that the water content in the reversed micelle system (water-alcohol) had no effect on the fatty acid synthesis. This was due to the fact that the water produced by the reaction partitioned to the water pools, because the lipase used for their experiment (from *Candida cylindracea*) has unusually amphiphilic surface and thus it was located in the micellar interface. This observation is opposite to the effect of water content on the equilibrium kinetics of hydrolytic reaction. However, this result provides information on the need for surface modification of enzyme functioning hydrolysis in a reversed micellar system.

3. Lignins and Lignin-Like Compounds

Aside from linear polyester synthesis, oxidoreductase enzymes can make naturally occuring lignin or lignin-like polymers. These enzymes include lignin peroxidase, Mn-peroxidase, laccase, xylanase, etc. [Chang and Dolphin, 1978; Lamport, 1986; Miyakoshi et al., 1996; Kurek and Monties, 1994; Farrel et al., 1989; Lehmann, 1981; Thomas et al., 1970; Kirk, 1987; Meunier, 1987; Meunier and Meunier, 1985; Wang et al., 1995a,b; Conroy et al., 1978]. Most of these enzymes require hydrogen peroxide or molecular oxygen as a *switching molecule* that has to be fed during the polymerization. Lignin peroxidase from *Phanerochaete chrysosporium* was used to perform the oxidation of aromatic compounds, including polycyclic hydrocarbons and heterocyclic compounds in organic solvent media [Vazquez-Duhalt et al., 1994; Farrell et al., 1989]. A microbial peroxidase, Novozyme 502, has been cloned and expressed in high yield and is now offered on an industrial scale [Novo product information, 1996]. The enzyme is able to polymerize the water-soluble part of the non-sulfonated and sulfonated lignin, suggesting its use as a "glue", instead of the conventional urea-formaldehyde and phenol-formaldehyde binder resins used for wood composites [Yde, 1992]. Lignosulfonate is a highly effective and economical adhesive polymer, acting as a binding agent or gap-filling glue. Due to sticky and hydrophilic properties, the use of lignosulfonate polymers has been tried on roads. When applied to a dusty road, lignosulfonate polymer has the ability to bind the dusty dirt particles together and form a hard, durable road surface (Wesco Technologies, LTD., San Clemente, CA, USA). Another usefulness of commercial lignosulfonate polymers comes from their dispersing, emulsifying, and binding properties.

Fig. 5 shows a peroxidase-catalyzed polymerization of kraft lignin (hydrophobic lignin) and lignosulfonate (hydrophilic lignin), where molecular weights in excess of 300,000 were obtained. Moreover, the use of organic solvent in the reaction media can change the property of the polymers such as molecular weight, thermal and mechanical characteristics, since the reaction with insoluble portions of the hydrophobic lignin increases with enhanced lignin solubility. There have been extensive research results which support the claim that horseradish peroxidase in organic solvents was able to oxidize lignins by phenoxy radical formation, allowing copolymerization with various phenols [Popp et al., 1991; Blinkovsky and Dordick, 1993]. These researches were focused on the development of novel series of polymeric materials (Table 1), i.e., lignin-incorporated plastics as extenders in polyurethanes, acrylics and epoxy resins. Since lignin is second to cellulose in abundancy on earth, these lignin-modification studies show great potential for use as a substitute for petroleum based plastics.

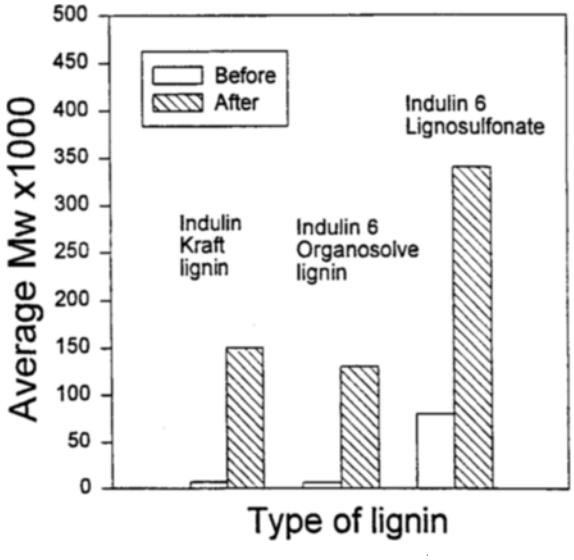

Fig. 5. Polymerization of kraft lignin and lignosulfonate using Novozyme 502.

Table 1. Thermal properties of kraft lignin and lignin copolymers

Copolymer	T_g (°C)	Decomposition temperature (°C)*
Lignin-phenylphenol copolymer	98.0	438
Lignin-cresol copolymer	119.7	425
Lignin-bisphenol A copolymer	178.5	433
Lignin (Kraft)	180.3	407

*Decomposition temperature at 30 % weight loss.

4. Phenolics and Aniline Polymerizations

Peroxidase-catalyzed oxidation of a variety of electron donors with hydrogen peroxide has been extensively studied using HRP [Bollag et al., 1995; Chance and Maehly, 1963; Dunford, 1991; Metodiewa et al., 1992; Miyakoshi et al., 1996; Harris et al., 1993a; Casella et al., 1992; Ortiz de Montellano et al., 1987; Cunningham et al., 1994; McDonald, 1989]. The enzyme requires a peroxy compound (e.g., hydrogen peroxide) as an oxidant [Nakajima and Yamazaki, 1987; Bollag and Liu, 1985]. The oxidative coupling of phenols can be achieved by the peroxidatic action. The mechanism of simple phenol oxidation by HRP is shown in Fig. 6. However, in the presence of excess hydrogen peroxide, some peroxidases including HRP and lignin peroxidase catalyze depolymerization reactions. The onset of depolymerzation was linked to the peroxidase compound-III formation and depended on the specific hydrogen peroxide: peroxidase ratio at a given phenol concentration [Joo et al., 1997]. This result can be applied to the development of peroxidase-catalyzed lignin degradation and the synthesis of high molecular weight phenolic resins.

While phenols give a complex mixture of products by ran dom coupling of resonance tautomers, *p*-cresol gave high yield of unique product such as Pummerer's ketone (yield: more than 45 %) [Brown, 1963]. The formation of dimeric or polymeric coupled products of aniline is attributable to nucleophilic attack of the amino nitrogen of a substrate on a quinoid intermediate. Various types of aniline derivatives were synthesized using HRP/H_2O_2 or HRP/O_2 [Holland, 1992; Cunningham et al., 1994; Pridham, 1963]. Interestingly, the oxidation of 2-naphthylamine and HRP leads to the formation of two polymeric products, according to the oxidation molecules (hydrogen peroxide or molecular oxygen). One is phenazine, another is an aromatic heterocyclic complex (Fig. 7).

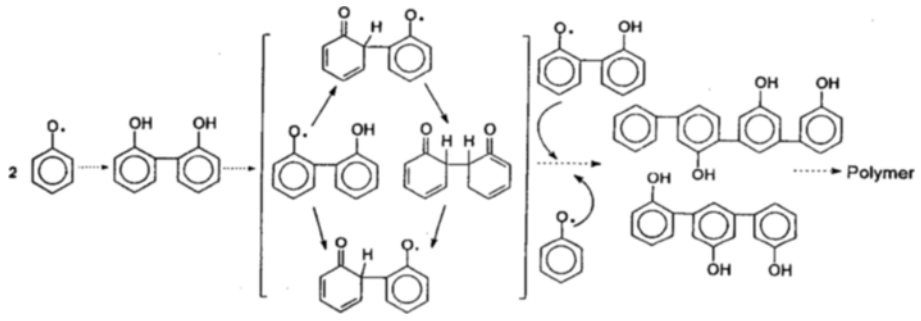

Fig. 6. Schematic representation of reaction mechanism for horseradish peroxidase catalyzed polymerization of phenol.

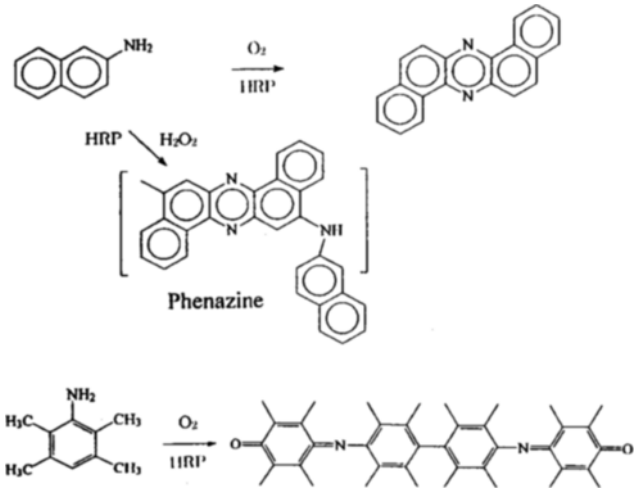

Fig. 7. Horseradish peroxidase-catalyzed macromolecular syntheses of 2-naphthyl amine and 2,3,5,6-tetramethylaniline.

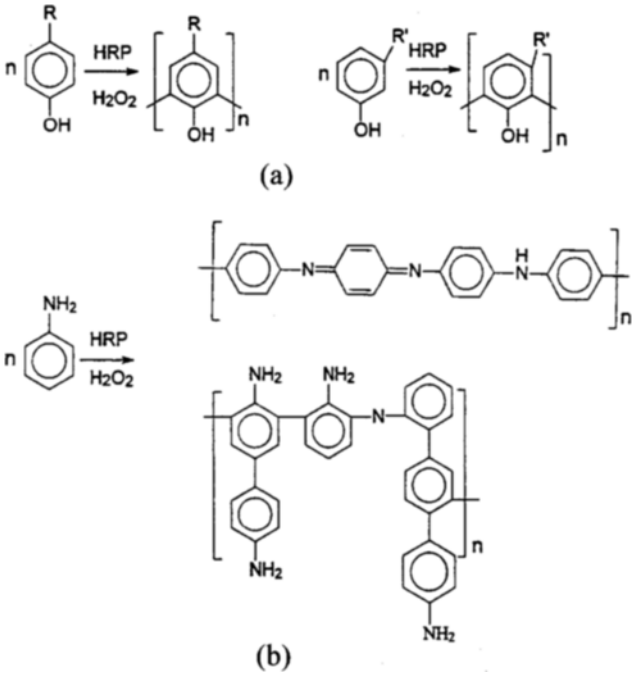

Fig. 8. Peroxidase catalyzed polymerization of (a) phenolics and (b) aniline.

Whereas, 2,3,5,6-tetramethylaniline gave aminoquinone type polymer, in the presence of HRP/O_2 (Fig. 7). But in the case of chloroperoxidase (CPO), unlike other peroxidase, the oxidizing potential is channeled into halide ion, which is then incorporated into an organic substrate: $AH+H_2O_2+H^++X^-\rightarrow AX+2H_2O$ [Thomas et al., 1970].

The use of a biocatalyst called soybean peroxidase or horseradish peroxidase enables the formation of formaldehyde-free phenolic resins (Fig. 8a), which is more environmentally benign and cost-effective than current industrial practices [Pokora and Cyrus, 1985]. Most phenolic resins are manufactured with formaldehyde, a toxic petrochemical. The formaldehyde acts as a "bridge" between phenol molecules in the polymer [Whitehouse et al., 1967]. To eliminate formaldehyde from the phenolic resin processing, some trials were conducted. Especially, the peroxidase-catalyzed enzymatic reaction allows phenol molecules to be linked directly together via a "free radical phenol coupling process" [Anni and Yonetani, 1992; Valoti et al., 1989; Takahama et al., 1992; Harris et al., 1993b]. This molecular structure (C-C linkage between phenols) is very important, since there are two main functional problems associated with phenol-formaldehyde resins. Phenol-formaldehyde resins are unstable and easily degraded to volatile organic compounds (VOCs) when exposed to high thermal conditions or ultraviolet rays. Emission of these toxic chemicals threatens human health and causes severe environmental problems [Clary et al., 1983]. Another benefit of this enzymatic polymerization of phenol is that it produces a much narrower range of molecular size in the resin polymer, resulting in more uniform polymer structures. This has the effect of reducing the viscosity of phenolic resin by as much as third order. Because of the reduction in viscosity, coatings using soybean peroxidase catalyzed resins can be formulated with lower solvent content [Prane, 1994].

The phenolic polymer synthesis using soybean peroxidase can be regarded as an "environmentally safe process" and may offer a variety of benefits to the manufacturer. Enzymol International Inc., a phenolic polymer producing company (Pokora, A., founder), claims that the process using this enzyme is milder and more efficient than conventional manufacturing processes [Enzymol International Inc. product information, 1996]. For example, formaldehyde-phenol resin synthesis generally occurs at temperatures of around 120-150 °C. However, the enzyme-catalyzed phenolic polymer resin synthesis requires only 60 °C condition, where the reaction completion time is

about 2- or 3-hour term, as compared to reaction times of 6-8 hours for formaldehyde-phenol resins. In 1981, although a similar approach on the phenol coupling using laccase enzyme was investigated by researchers at Union Carbide Corporation, they did not commercialize it, instead they reported the evidence of laccase-catalyzed phenol coupling [Schwartz and Hutchinson, 1981]. This means that the price of biocatalyst is also an important factor for commercial application. The price of laccase is much higher than that of peroxidase even though both enzymes perform the same reaction [Xu, 1996].

Aside from phenolic polymer synthesis, several kinds of phenolic polymerization using laccase enzyme have been reported. Laccase as well as peroxidase can perform oxidation of phenols, anilines, benzenethiols. Laccase (EC 1.10.3.1) are a family of multi-copper enzyme catalyzing lignin biosynthesis and degradation via oxidative and dehydrogenative reaction. A laccase-catalyzed oxidative synthesis of syringic acid to form poly(phenylene oxide) (PPO) was reported by Kobayashi and his co-workers [Ikeda et al., 1996]. PPO is widely used as an engineering plastic due to its high thermal stability, hardness and chemical inertness. Organic solvents affected both the yield and the molecular weight of the polymer. When the polymer solubility increased, both two properties also increased; in a solvent mixture, 2:1:3 (v/v/v) acetone/chloroform/buffer (acetate buffer, pH 5.0), the polymer synthesized by laccase showed highest molecular weight with 82% (g-polymer synthesized/g-monomer) yield.

Synthesis on urushi (oriental lacquer), a natural aesthetic durable coating material, has been well studied in Asian countries [Kumanotani, 1996; Vogl, 1996]. In Asia, wood furniture manufacturing companies use this urushi as a durable coating material, since the urushi shows excellent film properties with strong hardness. Urushi is an enzymatically polymerized product of urushiol from poison ivy or poison oak (*Rhus verniciflua Stokes*) [Zhang, 1992]. The curing of oriental lacquer consists of two-step processes. First is the phenol dimerization by laccase, and second is the autooxidative polymerization of the unsaturated side chains which are common in urushiol. Eight different kinds of identified urushiol (monomeric unit) are shown in Fig. 9 [Ishii et al., 1995]. To improve mechanical properties of urushi, the changes of the composition ratio and chemical binding states of the component element during the hardening process are also important. It was revealed that the oxygen related functional groups (e.g., C-OO, C=O, C-O-C, C-OOH, C-N, N=O) in the lacquer surface increased significantly as the oxygen content increased during the reaction [Iijima, 1996]. Since laccase performs concomitant reduction of molecular oxygen instead of hydrogen peroxide which is required for peroxidase action [Reinhammer and Malmstrom, 1981], such a result might be expected. Useful polymerizations, including natural pigment synthesis and redox-active polymer synthesis, were also performed by using peroxidase or multi-enzyme systems. Peroxidase-catalyzed melanogenesis, a process forming polymer pigments, is also an interesting polymerization for use in food and tanning processes. It was found that the peroxidase serves as an alternative to tyrosinase in the oxidative polymerization of 5,6-dihydroxyindols to melanin [d'Ischia et al., 1991]. Poly (quinone) is one of the interesting polymers for electronics applications. The polymer, the so called "redox-active polymer", is widely used as polymeric wire in circuit boards, battery electrodes, sensor plates, electrical polymer conductors, and anticorrosion/antioxidants [Diaz et al., 1987]. Wang et al. utilized two enzymatic steps to synthesize linear poly(quinone) having *o*-positional carbon to carbon (C-C) linkage [Wang et al., 1995c]. Initially, β-glucuronidase from bovine liver was used to catalyze the transfer of β-glucose to hydroquinone to give glucose-β-D-hydroquinone. At the second stage, peroxidase catalyzed polymerization was performed by using glucose-β-D-hydroquinone and resulted in water-soluble poly(hydroquinone). The polymer having molecular linearity with molecular weights ranging 1,600-3,200 g mol^{-1} showed good reversible redox behavior.

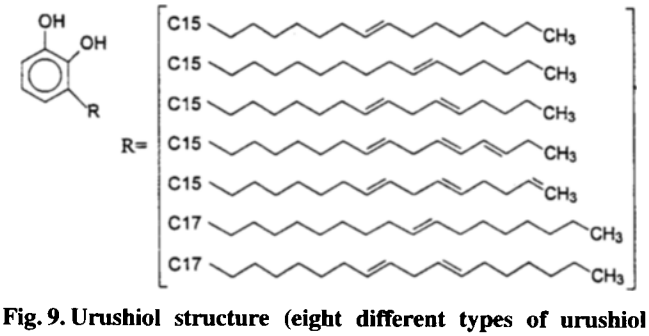

Fig. 9. Urushiol structure (eight different types of urushiol were identified).

Studies on the biochemical synthesis of polyaniline were performed (Fig. 8b) [Akkara et al., 1991]. The polyaniline (PANI) synthesis using horseradish peroxidase is important in polymer technology, since the polyaniline produced by the enzyme provides various industrially useful properties such as good solubility in water, excellent electrochemical conductivity, and superior non-linear optical properties, etc. [Rao et al., 1994]. Most of the chemically synthesized PANIs do not provide such solubility in water. Interestingly, polyaniline which was synthesized by the chemical method had a 10-times lower third order non-linear susceptibility value than the value obtained for the peroxidase-catalyzed polyaniline [Akkara et al., 1994]. Table 2 shows the third order non-linear susceptibility values of polyaniline and co-polyaniline by a degenerate four wave mixing (DFWM) experiment.

NEW STRATEGIES FOR SPECIALTY POLYMER SYNTHESIS

1. Chemoenzymatic Synthesis: High Productivity and Reaction Selectivity

While chemical catalysts provide high productivity, enzymes provide high selectivities. The combination, the so called "chemoenzymatic process", may compensate for their inherent shortcomings. Good examples are the syntheses of optically active prochiral-fluorine-substituted diester [Kitazyme et al., 1988], sugar-based poly(sugar-acrylate) [Chen et al., 1994], and glycosylglycerol-fatty-acid ester (GFE) [Ronchetti, F., Italy, *EU-PROJECT Programme*, Date No. 951101], etc. The prochiral, fluorine-substituted diester was chiroselectively pol-

Table 2. Non-linear optical susceptibilities for various polymers

Polymer	Reaction condition	pH	$\chi(3)^{\dagger}$ 10^{-11}esu	Yield (%)	References
Chemical synthesis	1 M HCl				
Polyaniline*	+ammonium persulfate at 2-4	4 °C	80	High	Akkara et al., 1994
Peroxidase-catalyzed reaction	$HRP+H_2O_2$ in various Solvent : buffers				
Polyaniline	DMF : HEPES (60 : 40)	7.5	740		Akkara et al., 1994
Polyaniline	DMF :		750	14.8	Akkara et al., 1994
Polyaniline	HEPES (50 : 50)	7.0	750		Akkara and Kaplan, 1996
		7.5			
Polyaniline	DMF : HEPES (40 : 60)	7.5	760	8.4	Akkara et al., 1994 Akkara and Kaplan, 1996
Poly(*p*-phenylphenol-Aniline)	DMF : HEPS buffer	7.5	2.5	90	
Poly(ethylphenol)	Isooctane		6.8		Akkara et al., 1994
Poly(dodecylphenol)	Isooctane : water		5.4		Akkara et al., 1994
C14PP/Phenol(1 : 10)/Langmuir Blodgett			100	Monolayer	Bruno et al., 1995

*chemically synthesized aniline has no primary and secondary amine stretching (N-H; 3,300 cm^{-1}, C-N; 1,307 cm^{-1}) and only shows C=N stretching.

†The third order non-linear susceptibility was measured by DFWM.

ymerized using cellulase from *Trichoderma viride* (TVC) in benzene after the non-enzymatic monomer preparation. GFE synthesis is very important in bioengineering applications. There is a growing demand for amphiphilic carbohydrate derivatives for new biological and biomedical applications, as nonionic surfactants required for the solubilization of integral membrane proteins. An example is the preparation of GFE-linked protein drugs such as GFE-asparagenase for the treatment of leukemia lymphoma patients. GFE is a compound of this type, in which a hydrophilic polyhydroxylated moiety is linked to one or more hydrophobic chains. The first step is the enzyme utilization towards a selective acylation : the regiospecific binding between glycerol subunit and 6'-sugar hydroxyl can be achieved using lipase. The second step is the chemical modification with other compounds to make several derivatives. Stereo-/regio- and regular-sbindings are not needed in this step.

Dordick and his research group at the University of Iowa (Iowa City, USA) incorporated sugars into the polyester backbone [Patil et al., 1991; Chen et al., 1995]. By using alkaline protease from *Bacillus* sp. (e.g., *Subtilisin carlsberg*) and Fenton's reagent (ammonium persulfate/H_2O_2/$FeSO_4$), poly(sugar-acrylate) could be synthesized in water/water-miscible solvent mixtures, resulting in hydrophilic, biodegradable polymers (Fig. 10). In their approaches, hydrogels were prepared from β-methylglucoside 6-acrylate with diacrylate (or sugar based acrylate) crosslinker. The gels adsorbed 300 times their weight in water.

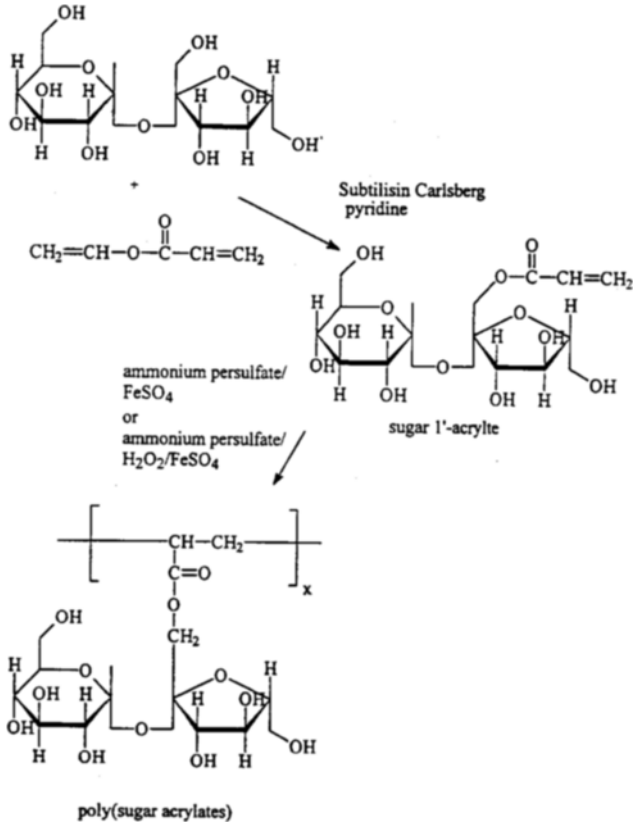

Fig. 10. Chemoenzymatic synthesis of a poly(sugar acrylates) [Patil et al., 1991].

2. Combined Multi-Enzymatic Polymerization

Generally, when multiple-enzyme systems are properly chosen, problems in positional and stereochemical polymer synthesis can be overcome as demonstrated by Wang et al. [1995c], in the case of multi-enzymatic synthesis of poly (hydroquinone). In their approach, the primal use of group transfer enzyme, β-glucuronidase, provides a means to prepare a unique monomer for subsequent enzymatic action. Recently, researchers at Scripps Research Institute in La Jolla, California, and Cytel Corp. in San Diego have developed a technique for solid-phase synthesis of oligosaccharides and glycopeptides [Schuster et al., 1994; Borman, 1994]. A combined chemical and multi-enzymatic procedure could provide a basis for an automated oligosaccharide and glycopeptide synthesizer. This technology is very useful for automated vaccine production.

3. Diversification of Biocatalytic Polymerization

In recent years, biocatalytic polymerization has been diversified. One example is the enzyme-mediated polymerization of phenolics on a Langmuir trough (LT) [Bruno et al., 1995]. The combination of peroxidase-catalysis and biomimetic Langmuir trough which resembles a whole cell membrane system could successfully provide an ordered and oriented monolayer aromatic polymer film, as compared to the phenolic polymerizations in bulk organic media (Fig. 11). In fact, the previous experiments in bulk solution had many limitations in practical applications, resulting in low thermal stability and non-linear susceptibility caused by branching, crosslinking and randomized coupling tendencies. Nevertheless, the LT assembled phenol polymerization showed more than 10 times higher non-linear susceptibilities.

It is also important to investigate the reaction diversities of currently existing enzymes, which offers another important polymerization reaction. As an example, a novel phenol-sucrose copolymer synthesis using peroxidase from horseradish was performed in alkaline pH (reaction condition: 50 % v/v dioxane/buffer, pH 11) [Joo, 1997]. Since the peroxidase shows pH-dependent reactivity toward both phenol and sugar molecules, it was possible to make inter-linkage between phenol and sucrose molecules via a cooxidative carbon to carbon coupling process. The surface tension of the synthesized polymer in water was 25.6 mN/m, which means that the phenol-sucrose copolymer has superior surface tension lowering nature in water. Some important biochemical and commercial surfactants including ionic- and non-ionic surfactants are summarized in Table 3. Among the commercial surfactants, non-ionic surfactants have the low surface tensions: $C_{10}H_{21}(POE)_6$-OH, γ=30.8 mN/m; p-t-$C_8H_{17}C_6H_4$-$(POE)_7$OH, γ=30.8 mN/m. Microbial biosurfactants including glycolipids, polysaccharide,

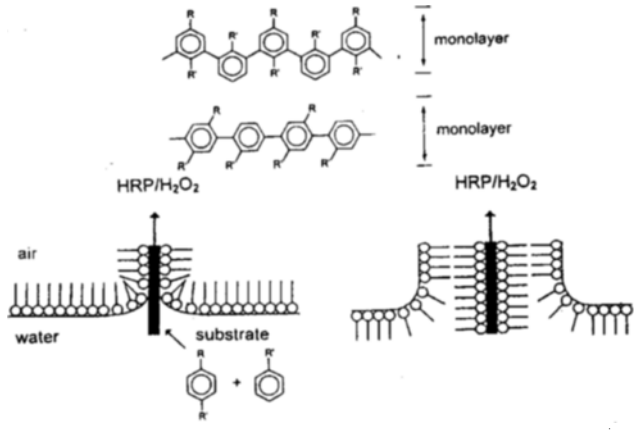

Fig. 11. Peroxidase-catalyzed phenol polymerization on a Langmuir Trough [Bruno et al., 1995].

Table 3. Surface tension of chemical and biochemical surfactants

Surfactants	Temp. °C	cmc/C_{20}	γ (mN/m)	References
Chemical surfactants				
$C_{12}H_{25}SO_4^-Na^+$	25	2.0	40.3	Myers, 1988
$C_{12}H_{33}SO_4^-Na^+$	60	2.5	37.8	Myers, 1988
$C_{12}H_{25}C_6H_4SO_3^-Na^+$	70	1.3	40.3	Myers, 1988
$C_{16}H_{33}C_6H_4SO_3^-Na^+$	70	1.9	45.0	Myers, 1988
$C_{12}H_{25}C_5H_5N^+Br$	30	2.1	42.8	Myers, 1988
$C_{12}H_{25}N(CH_3)_3^+Br$	30	2.1	41.8	Myers, 1988
P-t-$C_8H_{17}C_6H_4(POE)_7OH$	25	22.9	30.8	Myers, 1988
$C_nH_m(POE)_xOH$	25-30	6.3-17	>31.8	Myers, 1988
Biosurfactants from Microorganisms				
Glycolipids (Rhodococcus sp.)[1]	25	-	30	Georgiou et al., 1992
Rhamnolipid (Pseudomons sp.)[2]	25	-	29	Georgiou et al., 1992 Fiechter, 1992
Sophorolipids (Torulopsis sp.)[3]	25	-	33	Georgiou et al., 1992 Fietcher, 1992
Surfactin (Bacillus sp.)[4]	26	-	27-32	Fietcher, 1992
Enzymatic Synthesis using HRP				
Phenol-sucrose copolymer (Mw-1,000 g mol^{-1})	25	-	38	Joo, 1997
Phenol-sucrose copolymer[5] (Mw-1×10^4 g mol^{-1})	25	-	25.6	Joo, 1997

*Range; n=12-16, m=21-33, x=6-15

[1,2,3,4,5]Yield; glycolipids: 660 mg $liter^{-1}$, Rhamnolipid: 2.5 g $liter^{-1}$, Sophorolipids: 38-77 g $liter^{-1}$, Surfactin: available, Phenol-sucrose copolymer: 280-400 g $liter^{-1}$.

phospholipids, fatty acid, and peptide oligomers show much lower surface tension than the value of chemical surfactants. The most widespread microbial surfactants are glycolipids. To date, the "*surfactin*" in Table 3 from *B. subtilis* is known as one of the most powerful surfactants. The surface tension of the linear peptide, FA-$_L$Glu-$_L$Leu-$_D$Leu-$_L$Val-$_L$Asp-$_D$Leu-$_L$Leu, is 27.2 mN/m. Since the phenol-sucrose copolymer synthesized by peroxidase satisfies some of the requirements for surfactant such as amphiphilicity, surface tension lowering nature, and foam boosting [Medina, 1996], it can be used for particular purposes such as biosurfactants and separation column materials. It is also worth noting that for the latter application the sugar-based adsorbents are expected to reduce protein adsorption [Dordick et al., 1994]. Some enzymes have been also found to acrylate sucrose by condensation reaction. However, such condensation catalyzing enzymes in general do not provide phenol to phenol coupling and phenol to sugar coupling reactions. Only the peroxidase from plants can perform these kinds of reactions; "hemicellulose in wood is a naturally occurring phenol-sugar polymer".

CONCLUDING REMARKS

Progress toward the synthesis of specialty polymers is being made from different approaches. Employment of lipases, esterases, transferases, and oxidoreductases for the preparation of highly functional polymers will continue as one area of a promising technology for polymer synthesis. The needs for specialty polymers will lead to many unexpected findings of reaction diversities given by biocatalysts. One of the most compelling evidences for the validity of the concept of macromolecular synthesis is that biocatalysts in living organisms already synthesize many of these polymers man-made. Therefore, the examination of naturally occurring biological macromolecules or their synthetic system yields other useful lessons. Orb-web spinning spiders synthesize a hygrogenic silk protein performing so called "supercontraction process" [Lewis, 1992]. Remarkably, the polymer has a tensile strength 16 times greater than that of nylon [Robson, 1985]. The key process exhibiting such a polymer property is the supercontraction; 60% contraction of length and thousand-fold decrease of elastic modulus have occurred from original silk protein. However, man-made polymers do not exhibit such a supercontraction as spider silk does in water-fibroin solution. A similar silk protein is also produced by silkworms (e.g., *Bombyx mori*) [Marsh et al., 1992]. The amino-acid sequences of *B. mori* fibroin consist of two repeat units characterized as crystalline domain (1-2 kbase) and amorphous domain (220 bp). The polymers produced are extremely monodisperse and exhibit many useful industrial application features such as good abrasion resistance, high tensile strength, and unusual electromagnetic resonance in ultraviolet rays [Craig, 1989; Warwicker, 1960]. Notably β-keratins contain an abundance of the identical repeating sequence, Ser-Gly-Ala-Gly-Ala, which indicates a highly crystalline structural analogy [Robson and Garnier, 1988]. The fibroin synthesizing gene is located in the posterior region of the silk grand and the entire synthetic process including genetic map is now easily obtainable elsewhere [Tsujimoto and Suzuki, 1979; Tsuda et al., 1979; Gage and Manning, 1980]. In nature, some kind of amino acid sequences show interesting features. A sequence of Ala-Ala-Thr has been known as the structural building unit of fish anti-freeze glycoproteins, depressing the freezing point of water [Robson and Garnier, 1988; Feeney et al., 1986]. The emergence of hydroxylated proline in collagen is related to the thermal stability of the collagen towards thermal denaturation at high temperature [Lehninger et al., 1993].

To summarize, there has been a tremendous growth of interest in the field of "polymers from biocatalyst" both in academia and in industry. While much remains to be done, transformations based on enzymatic catalysis are now providing valuable tools for specialty polymer synthesis. The advantages of using biocatalysts include fast reaction rates, at mild reaction condition, and high yield of products with high reaction selectivity. However, examples of industrial polymerization processes using enzymes as catalysts have been scarce so far, because economic and engineering hurdles may impede their introduction to an industrial scale. Since many of these polymers are in the early phases of development and the research requires state-of-the art technology, it is difficult to determine whether mass production will be able to bring down their high production costs. In spite of these problems, there is a strong demand in industries and a number of significant technical developments have flourished. Moreover, steady progress in the science of enzymology promises that the inaccessibility of many enzymes can be reduced. Compared to the alternative chemical synthesis available, biocatalyst-mediated polymer synthesis also will be a new process for the 21st century.

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