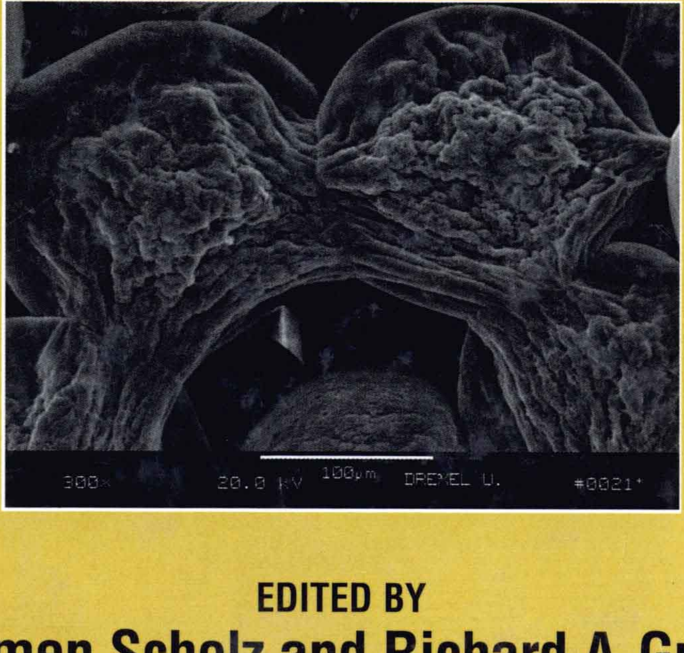
ACS SYMPOSIUM SERIES 764

Polymers from Renewable Resources

Biopolyesters and Biocatalysis



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Polymers from Renewable Resources

Foreword

THE ACS SYMPOSIUM SERIES was first published in 1974 to provide a mechanism for publishing symposia quickly in book form. The purpose of the series is to publish timely, comprehensive books developed from ACS sponsored symposia based on current scientific research. Occasionally, books are developed from symposia sponsored by other organizations when the topic is of keen interest to the chemistry audience.

Before agreeing to publish a book, the proposed table of contents is reviewed for appropriate and comprehensive coverage and for interest to the audience. Some papers may be excluded in order to better focus the book; others may be added to provide comprehensiveness. When appropriate, overview or introductory chapters are added. Drafts of chapters are peer-reviewed prior to final acceptance or rejection, and manuscripts are prepared in camera-ready format.

As a rule, only original research papers and original review papers are included in the volumes. Verbatim reproductions of previously published papers are not accepted.

ACS BOOKS DEPARTMENT

Preface

The meeting of the Bio/Environmentally Degradable Polymer Society was held for the 7th time in a series in August of 1998. The conference was held in a timely and locally conjunction with the symposium on Biodegradable Polymers held at the 216th American Chemical Society (ACS) meeting, which enabled us to bring together scientists from industrial, academic, and governmental agencies and to discuss the most contemporary environmental biopolymer issues.

In the search for a polyester-based structural material, Carothers at DuPont developed Nylon 66 instead in 1936. Polyesters with molecular weights high enough to be useful as fibers were introduced a few years later. Today, polyesters based on poly(ethylene terephthalate) and ethylene glycol are part of our daily life in form of soda bottles, fibers, and bases for photographic films and magnetic tapes, to mention just a few. Aliphatic polyesters have been recognized for their biocompatibility and their susceptibility to hydrolytic degradation. Whereas current research on structural, aromatic polyesters focuses mainly on processing and engineering issues, aliphatic polyesters gain increasing consideration as biomedical materials. In particular, poly(lactic acid) (PLA) and poly(glycolic acid) (PGA) are the polymers of interest, for implants, drug delivery systems, and scaffolds, because they are biocompatible, degrade within about one month upon implantation, in addition, they are FDA-approved materials. Polymer research constantly develops and probes new routes to the synthesis of polyesters, new catalysts are found, reaction mechanisms are understood in more detail, and efforts focus on tailoring physical properties. In addition to their biocompatibility, PLA, PGA, and copolymers thereof, exhibit mechanical properties, which make them uniquely suited for applications in sutures, staples, screws, clips, fixation rods, and drug delivery systems. High strength, thermoplasticity, and non-toxicity are inherent properties of PLA and PGA, which are complemented by the controllability of the crystallinity, hydrophilicity, and therefore overall degradation rate.

Polyhydroxyalkanoates (PHAs) are polyesters that occur in nature. They are produced by a wide variety of microorganisms as an internal carbon and energy storage, as part of their survival mechanism. Polymer production is triggered when the environment changes to unsuitable living conditions. The storage polymer is intended to sustain the survival of the organism when one or more nutrients become unavailable. Depolymerase enzyme are expressed and degrade this stored polymer. PHAs and in particular poly(β-hydroxybutyrate) (PHB), poly(β-hydroxyvalerate) (PHV), and copolymers thereof have been considered as alternatives to conventional fossil fuel-based bulk polymers. The properties of PHB and PHBV are comparable to polypropylene. Pros and cons of the use of microbially derived commodities are subject to scientific as well as political debates and are reflected in the current volume.

The quest for new materials and environmentally sound procedures drives research to new frontiers, one of which brought natural and synthetic polyesters closer together by using enzymes, natural products, as catalysts for polyester synthesis. This novel approach to environmentally suitable materials makes the terms "synthetic" and "natural polyester" obsolete, creating a new class of materials, polymers "from and through renewable resources". Biocatalytic reactions combine several advantages, their site-specificity is most important for stereoregular reactions and prevent effectively the formation of side-products. Enzymes can be easily recovered after the reaction and can in average go through about 100 cycles of usage. Most importantly, ways to use enzymes in organic solvents have been discovered, opening new avenues for organic (polymer) synthesis. Although the use of enzymes as catalysts in organic reactions is a relatively new branch of chemistry, extraordinary achievements in enzyme catalysis are reported in this publication. Collaborations between enzymologists, engineers, and chemists will carry the field of biocatalytic synthesis to a new level within the near future. This book comprises 21 chapters related to research being pursued on the forefront of biopolyesters and enzymatic catalysis.

The editors thank all contributors to this publication and wish them success in their ongoing pursuit for novel materials and advanced technologies that contribute to better the world around us.

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Biopolyesters and Biocatalysis Introduction

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Recently, there has been great interest by both academic and industrial scientists to develop polyesters that provide an environmental benefit. Such polyesters, denoted herein as 'biopolyesters', might be synthesized from readily renewable resources, by methods such as biocatalysis that by-pass non-desirable toxic chemicals, and that degrade and are mineralized after disposal. Most of the polyesters used today are obtained by classical chemical synthetic techniques. As described in this book, a new arsenal of biocatalytic strategies is under study that provides advantages including all aqueous processes, enantio- and regioselective transformations, the use of non-toxic catalysts, one-pot multistep transformations and efficient conversions of renewable resources to value-added products.

Background

The term "Biopolyester" can be understood in several different ways. Biopolyesters can be interpreted as polyesters of strictly biological origin. One can interpret biopolyesters also as polyesters that have been synthesized by biological means, for instance by enzyme-catalyzed polymerization reactions. Moreover, there are hybrids between these two strict definitions of biopolyesters. For example, monomer synthesis for poly(lactic acid), (PLA), is by a biological process in which lactic acid is produced microbially by the fermentation of a renewable, polysaccharide-based resource, mostly corn. Lactic acid is subsequently polymerized chemically into PLA by a condensation reaction.

Polymers preferred by nature seem to be polyamides, in biological terms proteins, and polysaccharides. Proteins fulfill in biological systems catalytic functions (enzymes), regulatory functions (peptides), and serve as transport vehicles. In addition, proteins form structural and storage units and have defensive functions. Polysaccharides are the other large group of natural polymers. They exist in the form of cellulose, starch, alginate, glycogen and many others. All of the above mentioned polysaccharides fulfill structural and storage functions. The most significant, life sustaining biological polymers, DNA and RNA, are polynucleotides. Using chemical nomenclature, polynucleotides are poly(phosphodiesters). Their backbone is composed of ribose and phosphoric acid, which are linked by phosphodiester bonds. This ribose-phosphate-ester linkage is the only covalent bond that connects the four nucleic bases in their specific order. DNA and RNA are recognized for their extraordinary function attributes, which are based upon the nucleosides and their order of arrangement. Therefore, they are classified as polynucleotides rather than polyesters.

Truly naturally produced, high molecular weight biopolyesters exist only in the form of poly(hydroxyalkanoates), (PHAs). PHAs, just like natural rubber, hold an "outsider" position in nature. PHAs are polyesters while rubber is a natural polyolefin, both of which seem to be the product of one of nature's exotic moods, down a playful alley off the common track. Lemoigne[1] first discovered PHAs in 1925 while he was examining *Bacillus megaterium*. PHAs then fell into oblivion for the next couple of decades. The world was taken by storm by synthetic polymers. Staudinger[2] had elucidated and forcefully presented the physical structure of polymers, showing that polymers are covalently linked molecules rather than agglomerates of small molecules. Polymer research gained momentum over the next several years, driven by scientific inquisitiveness and the quest for new materials as well as by the machinery of the Second World War. Plastics can be made for all different kinds of applications with specific, almost made-to-measure properties. There seemed to be no limit to the development of new polymers with properties mimicking the strength of steel, the softness of cotton and everything in between. Polyisoprene superceded natural rubber, giving the same kind of boost to the automobile industry that Nylon 66 gave to the fashion industry. Natural polymers were thrust into the background. The euphoria over synthetic polymers started to wane as a result of the oil crisis in the 1970s. The exhaustibility of fossil fuels was suddenly recognized. Energy conservation, recycling, reusability and the search for new alternatives and renewable resources became significant issues. In addition, environmental concerns and a developing sense of environmental responsibility evolved at about the same time, demanding environmentally sound production processes, that would reduce pollution, improve waste management, and provide biodegradable consumer products. In short "green" Zeitgeist now took the world.

In Europe, where population density is high and land area is much more utilized than in North America, the "Green Movement" developed faster. More focused, "green" alternative groups developed politically and rather quickly into reputable "green parties". Several of these "green parties" share governmental power throughout Europe today.

Therefore, it is not surprising that the first commercial facility for the production of fully biodegradable microbially produced polyesters was established by ICI[3] in England. Poly(β-hydroxybutyrate-co-valerate), (PHBV), was produced from glucose and propionic acid by microbial fermentation of *Alcaligenes eutrophus* (now renamed *Ralstonia eutropha*) and marketed under the tradename Biopol^{TM}. One of the first commercial products made from PHBV were shampoo bottles marketed by Wella AG, Germany. Biodegradability is viewed by some as one of the most important assets of biopolyesters. Upon disposal in a biologically active environment, biodegradable biopolyesters can serve as a food source for numerous microorganisms, including fungi, bacteria, and algae. Degradation rates of PHBV are generally rapid in a wide variety of disposal environments including soil, compost and marine ecosystems. High production costs and/or an inability to achieve suitable performance criteria have thus far been obstacles to the commercialization of many biodegradable polymeric products. Thus, despite the biodegradability of some biopolyesters, this property attribute has not been sufficient to allow these products to gain a large share of the plastics market. Even though customers in European countries are more concerned with environmental issues and are, in general, more willing to pay a higher price for an environmentally sound product, commercial success is still governed by a need to reach a suitable cost-performance profile. As for the case of the shampoo bottles; the bottles were more expensive than the shampoo they carried!

Research into biopolyesters, and biocatalytic routes to these polymers, is a broad field that often requires the involvement of interdisciplinary research teams. For example, biologists, chemists and polymer scientists need to communicate and conduct research in a coordinated manner to ultimately achieve a positive outcome. This characteristic of the field will be reflected in the research described in the following chapters. The remainder of this chapter will provide a summary of prominent biopolyesters that are currently in use or are the subject of much research.

Structure of Biopolyesters

All polyesters, of natural and synthetic origin, are characterized by the following common formula, see Figure 1.

Many aliphatic polyesters are obtained by lactone ring opening polymerization, see below. The most prominent representative is polycaprolactone (PCL), (R= $(CH_2)_5$), which is commercially synthesized by ring-opening polymerization of ε-caprolcatone, see Figure 4, using metal oxides as catalyst.[4] Some of the earliest reports on the biodegradation of aliphatic polyesters were performed by using PCL.[5,6] Based on its biodegradability, PCL finds primarily applications as blown films for food and yard compost bags and matrix systems for controlled and slow release of pesticides, herbicides and fertilizers. Based on its low melting point (about 60 °C), excellent melt formability and high rigidity in the solid state, PCL has been successfully introduced as plaster replacement in orthopedic and orthotic applications. Due to its low glass transition temperature PCL is often used as additive for other, more brittle polymers. Even though the formation of miscible blends is rather rare in polymer science, PCL is miscible with a variety of polymers. For example, in blends of poly(vinyl chloride) with PCL, the latter acts as plasticizer, thus generating a processable material by reducing the modulus of poly(vinyl chloride).[7] It is equally important that PCL adds biodegradability to the material. Upon disposal in a landfill or compost facility PCL will biodegrade leaving behind the now porous polyolefin bulk material. Since plasticizers can make up to 30 weight% of the material, the use of biodegradable additives contributes significantly to a cleaner environment.

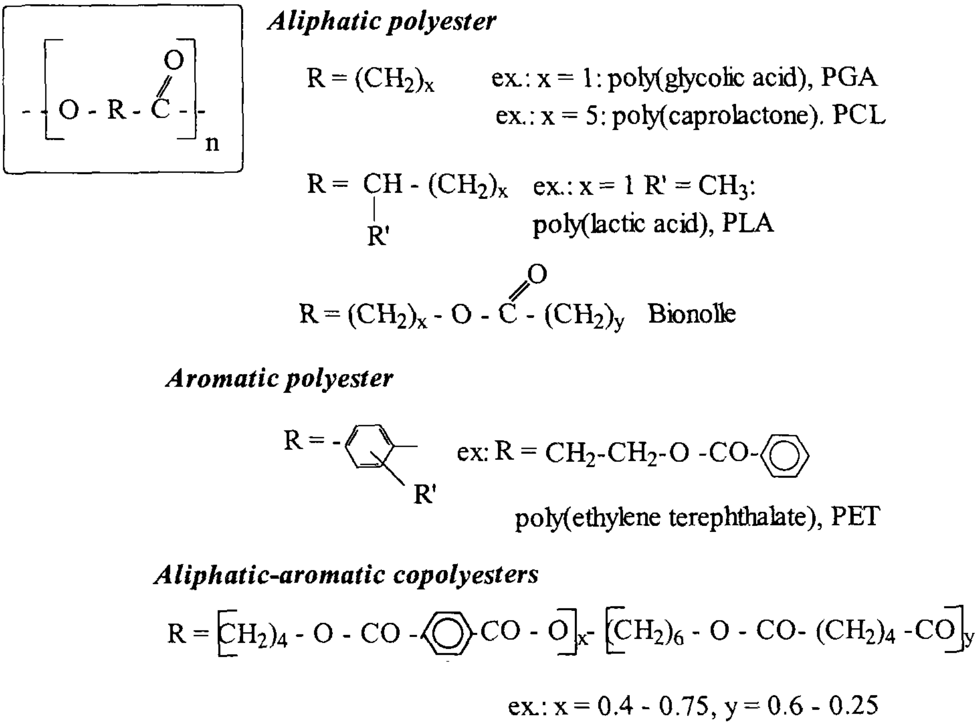

Figure 1: General Formula of Polyesters: Depending on the structure of R, polyesters are divided into aliphatic and aromatic polyesters

Bionolle is a family of aliphatic polyesters that has been developed and is now commercialized by Showa High Polymer Co., Ltd. in Japan. Bionolle polymers are structurally different from PCL, in that they are the product of a condensation polymerization of an aliphatic diol with a dicarboxylic acid. Using a variety of different diols and dicarboxylic acids leads to the possibility of fine-tuning the physical properties of the material. Isocyanate moieties are commonly added for structural rigidity and toughness. Bionolle is the Japanese counterpart to PCL. It is also biodegradable in soil, active sludge and in compost facilities. As for PCL, biodegradation results in the formation of carbon dioxide, water and biomass and no intermediate metabolism products are released that could be harmful to the ecosystem. Hence, Bionolle is used in similar types of application as described above for PCL.

Poly(lactic acid), PLA, and poly(glycolic acid), (PGA), are aliphatic polyesters that readily degrade by chemically-induced hydrolysis under physiological conditions. Therefore, PLA, PGA and their respective copolymers have found broad application as bioresorbable sutures, implants and drug delivery systems.[8,9] They are synthesized from their respective cyclic dimers, usually by ring-opening polymerization, see Figure 2.

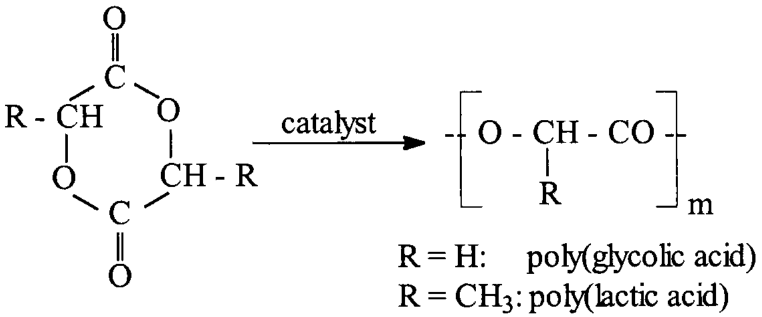

Figure 2: Ring opening polymerization of lactides

Lactic acid is obtained by the fermentation of engineered microbes of the genus *Lactobacilli.* These microorganisms are highly efficient sources of lactic acid. *Lactobacilli* can be subdivided into strains that produce either the L(+) isomer or the D(-) isomer. Fermentation has been optimized to achieve high yields in which batch processes give an average 2 g/L lactic acid per hour. A variety of different sugars are used as carbon sources in the fermentation process. These sugars are either specifically prepared enzymatically from starch for lactic acid production, or are by-products from fruit processing. In addition, lactose, a by-product in the cheese industry, can also be used as a carbon source for lactic acid production. In all cases sugars from renewable resources are transformed into a value-added product by an enzymatic whole-cell catalysis process. The subsequent steps in the production of PLA are based on synthetic chemistry and involve the formation of the dimer by a self-condensation reaction that yields a low molecular weight prepolymer. Depolymerization of the prepolymer yields lactide, which is then polymerized through ring-opening polymerization. The prepolymer can also be polymerized into high molecular weight PLA by the action of chain coupling agents.

In contrast to lactic acid, glycolic acid is produced on an industrial scale by a chemical process. Glycolic acid is present in small amounts in a wide variety of fruits and vegetables. It accumulates during photosynthesis in a side path of the Krebs cycle. So far, economically viable methods to produce glycolic acid in photosynthetic biological systems do not exist. On an industrial scale, carbon monoxide, formaldehyde and water are reacted at elevated temperature and pressure to yield glycolic acid. The acid is thermally converted into its dimer, which eventually is polymerized by a ring-opening polymerization.

More recently, aliphatic-aromatic copolyesters gained much commercial consideration. These copolyesters are synthesized by a condensation polymerization from butanediol and terephthalic acid, as the main components. To tailor the processability and to enhance biodegradation, these copolymers are further modified by copolymerizing with linear dicarboxylic acids (e.g. adipic acid) and glycol components with more than four methylene groups (e.g. hexanediol). One example for an aliphatic-aromatic copolyester is depicted in Figure 1. These copolymers were initially found useful as adhesives.[10] To enhance the environmentally benevolent aspect of these materials and to broaden the range of their use, aliphatic-aromatic copolyesters blended with cellulose esters have been processed into useful fibers, films and molded objects.[11,12] Biodegradation of aliphatic-aromatic copolyesters has been demonstrated in compost facilities.[13] *Actynomycetes*, which are gram-positive bacteria, have been identified as the major microbial species capable of degrading these copolyesters. The formation of aliphatic-aromatic copolyesters using enzymes as catalysts receives currently much consideration. Under tailored conditions, the use of lipases as catalysts for the synthesis of aliphatic-aromatic copolyesters led to polymers with an extremely low polydispersity. Lipases have also been successfully employed as catalysts in studies demonstrating the feasibility of conducting the polymerization in supercritical fluids.

To the best of our knowledge, the only high molecular weight biopolyesters that are currently produced from renewable resources by a whole cell biocatalytic process are poly(hydroxyalkanoates)[14], see Figure 3. PHAs are produced by a wide variety of bacteria and serve as a carbon and energy storage site for microorganisms. Under optimum fermentation conditions more than 90% of the cell dry weight may consist of PHAs. These biopolyesters are produced by the concerted action of three enzymes that include β-ketoacyl-CoA thiolase, acetoacetyl CoA reductase and P(3HB) polymerase. The polymerase functions to convert bioproduced monomers to high molecular weight polymers. Biopolyester synthesis by these organisms is triggered when the environment of the microorganism changes to unsuitable growth conditions, that is the lack of one or several nutrients or oxygen depletion for aerobic species.

Most PHA-accumulating bacteria produce poly(β-hydroxybutyrate), PHB, the PHA with a methyl side chain repeat unit, see Figure 3. Microbial PHB has a very high molecular weight ranging between 200, 000 and 500, 000 Dalton. Numerous depolymerase enzymes exist in a wide variety of environments that recognize the unique structure of these polymers. Specifically, the polymers are isotactic by virtue of having enantiopure β-substituted β-hydroxylalkanoate repeat units in the (*R*)-configuration. Thus, these polymers are readily degradable upon disposal as long as they are placed into environments with water and a diverse natural microflora.

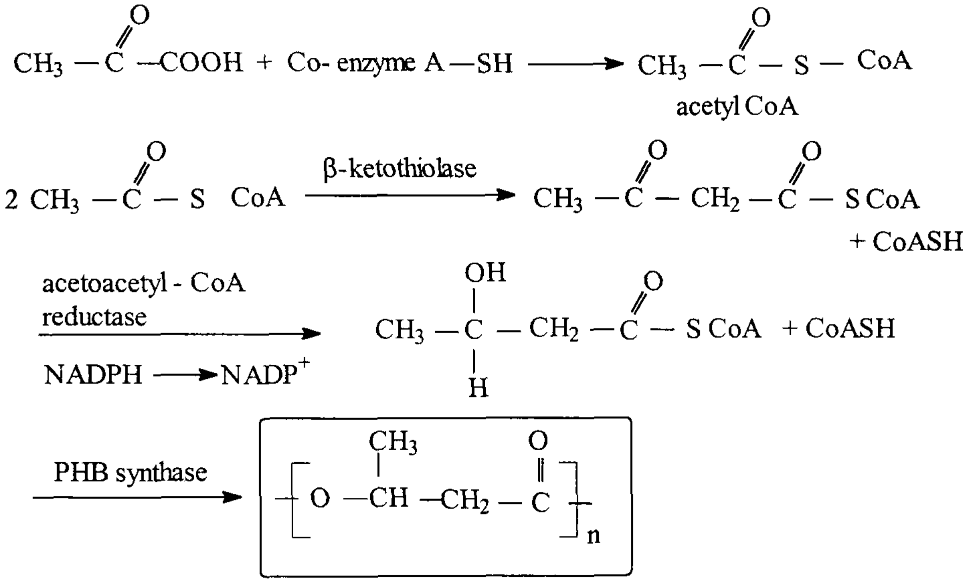

Figure 3: Schematic presentation of the enzymatic synthesis of PHB

PHAs can also be obtained by ring-opening polymerization of their respective lactones as is typical for the synthesis of a variety of polyesters, e.g. PCL, see Figure 4. The ring-opening polymerization of β-butyrolactone results in the formation of PHB synthetic analogs. Judicious selection of the catalyst can drive the reaction to yield a highly isotactic polymer. However, without the tedious synthesis of the enantiopure monomer, chemical synthesis fails to provide a complete stereoregular product that has only the natural configuration. It is noteworthy that the synthetic route does provide options for stereochemical variation. The synthesis of stereocopolymers, provides an opportunity to 'tailor' the functional properties of these products. Furthermore, synthetic routes have led to the synthesis of a predominantly syndiotactic PHB that has very different properties than its natural analogue.

Molecular weight and stereochemical control of polyesters synthesized by lactone-ring-opening polymerization is dependent on the catalyst used. There exists a