Biotechnological Polymers

MEDICAL
PHARMACEUTICAL
and INDUSTRIAL
APPLICATIONS

A CONFERENCE IN PRINT

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Biotechnological Polymers

a **TECHNOMIC** publication

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BUSINESS AND REGULATORY CONSIDERATIONS

BIOTECH POLYMERS:

WHAT THEY ARE AND WHAT THEY DO

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ABSTRACT

In the broadest sense, biotechnology can be defined as the use, modification or mimicing of naturally-occurring materials. On this basis, the earliest polymers used by mankind came from what we now call biotechnology. Included among these biotechnology-derived polymers are such standard materials as: cotton, wool, paper, rubber, shellac, leather, cellulosic derivatives, starch, and casein plastics or adhesives. In addition, the early paint industry relied on natural monomeric drying oils which oxidatively polymerized into paint films. Even though synthetic polymers began to dominate the field with the increasing availability of petrochemicals, many of these biotechnology-derived polymers are still widely used today. Current concerns about biodegradability and long-range renewable resources make biotechnology-derived systems even more inviting today. In addition, some "new" kinds of polymers are emerging, including chitin and chitosan, hyaluronic acid, elastin-like polymers, bioadhesives, melanin, and spider silk mimics, which show promise as economically viable materials for basic polymer applications as well as in cosmetic and medically related uses.

INTRODUCTION

We can define biotechnology in several ways, but here we will define it as the use, modification or mimicing of naturally-occurring materials. It's a matter of historical record that the earliest polymers used by humans came from what is now called biotechnology. Several currently established polymeric materials are among these time-honored biotech-derived polymers, including casein plastics or adhesives, starch and cellulosic derivatives, textiles such as cotton or wool, paper and wood products, rubber, shellac and leather. The early paint industry relied on naturally-derived drying oils which are monomeric materials that oxidatively polymerize into paint films. Current concerns about biodegradability and long-range renewable resources make biotechnology-derived systems even more inviting today. As we became more knowledgeable about the chemistry and nature of biological systems, we learned to adapt more biosystems for our use and benefit. For this reason, some innovative

types of polymers are emerging, including chitin and chitosan, hyaluronic acid, natural polyesters, elastin-like polypeptides, bioadhesives, melanin, and spider silk mimics, which display potential as economically viable materials in cosmetic and medically related uses and, perhaps, for some more basic polymer applications as well. The use of biotechnology in polymers is clearly evident in several recent books.[1,2,3]

TRADITIONAL BIOTECH POLYMERS IN RETROSPECT

Because reviewing polymer history goes beyond our scope, we'll concentrate instead only on biotechnology style polymers; several good polymer history reviews already exist.[4,5] Most early plastic, adhesive and textile applications used modifications of polypeptides or polysaccharides, although a few other natural polymers received a share of the market. Shellac, long used in coatings, is a natural polyester manufactured from excretions of the insect *Laccifer lacca*. While not recognized as polymeric materials until comparatively recent times, these natural polymers proved to be excellent resources. The tanning industry seems to be the first large-scale example of intentional polymer modifications which were designed to improve the properties and long-term utility of materials, although tanners had practically no comprehension of chemistry. In addition, vulcanization was developed by Goodyear and others with essentially no recognition of the macromolecular nature of rubber, much less the concepts of crosslinking. This lack of knowledge did not prevent widespread usage of these materials. Natural polymer modification soon became the basis for large industries as chemists and entrepreneurs worked with the best available materials. These natural polymers most often included cellulosics, natural rubber and casein.

Still, virtually all this progress occurred with negligible chemical background and no polymer theory. Cellulose was nitrated, acetylated, or esterified without anyone perceiving the macromolecular nature of the raw material. These modified polymers became useful plastics and coatings, and several cellulosics remain commercially important materials, with production in tonnage quantities every year. This list includes the textile and plastic uses of cellulose acetate and the various forms of rayon, various ether cellulosic derivatives, which are widely used in cosmetic and food products,[6] and sodium carboxymethylcellulose (CMC), which is used in most synthetic laundry detergent formulations to prevent soil redeposition. Cellulose itself can be converted into many low molecular weight materials via acid or enzyme hydrolysis, or into sugars via fermentation.[7] Although we seldom do, wood applications could be considered as polymer usage. In a similar manner, starch was used in the coatings industry long before the amylose structure was elucidated. As expected by its low cost, starch still finds widespread usage as coatings for paper and textiles. Naturally, these applications are dwarfed by the food consumption of starches, but many other natural polymers are less valuable as a food stock.

WHAT CAUSED THE PREVIOUS CHANGE FROM BIOTECHNOLOGY POLYMERS?

As we noted above, chemists and technicians worked with the best available polymeric materials they could find. This was usually natural products and their derivatives. In the early 1900s the basic thrust of the chemical polymer industry began to change as coal tar derivatives and petrochemicals became more available. Notwithstanding the fact that the first synthetic polymers were made in the late 1830s, these remained laboratory curiosities, or annoyances, for many years. Aside from the fact that the early chemists didn't perceive uses for them, the raw material supply was limited. Some synthetic polymers did achieve success in the coatings field where natural, monomeric drying oils were oxidatively polymerized into paint films. In a sense, these were the first synthetic polymers, derived from monomeric materials, instead of the phenolics, but the latter remain the first totally synthetic commercial polymers.

With the readily available raw materials derived from petroleum or coal, chemists began making commercial scale synthetic polymers. The success of these synthetic polymers led to an understandable decrease in the emphasis on natural polymers. The synthetic polymer molecules were much simpler which made them easier to understand theoretically. The natural polymers were eventually found to be extraordinarily complex in both structure and biosynthesis. Chemists found it far easier to deduce structure-property relationships for the synthetic polymers. A third factor impelling and propelling the synthetic polymer revolution was the ease of creating a broad range of properties, which seemed impossible with the natural polymers.

In the last few decades, some additional factors have permeate our deliberations which could redirect attention back toward natural systems. These factors include: (1) long-range renewable resource considerations and (2) biodegradability. The natural petroleum and coal reserves are not unlimited. Many disparate chemicals, such as medicines, also depend on this supply, in addition to uses as fuels. While biologically-derived, petroleum and coal are not considered renewable resources. Add to this the obvious fact that most petroleum based polymers either do not biodegrade, or show very limited degradation, with potentially major waste disposal problems. While recycling could partially alleviate this obstacle, the economics of recycled plastics seem uncertain. Most currently available recycled plastics carry a premium price making virgin plastics use more inviting. Unless petrochemicals increase in price, or decrease in supply, it's unlikely that recycling will solve these problems completely. The fact than many natural polymers biodegrade fairly readily provides yet another impetus for the re-examination of these materials. It makes much long-term, economic sense to do this before petrochemical supplies dwindle or become restricted by uncontrollable factors, such as war or political uncertainties.

Although a return to the old style polymers, such as casein plastics, cellulose nitrate and cellulose acetate, may not be appealing, because of their severe deficits, some of the newer materials have properties that are actually better than anything we can make from petrochemicals. Everyone knows that wool is eaten by moths and other insects, that cotton shrinks and doesn't hold a crease (unless treated with another polymer), that cellulose acetate is solvent sensitive, and that cellulose nitrate

is highly flammable. In the past few decades we have learned much significant information which enables us to develop new and better polymers from biotechnology; polymers with better properties than those we now prepare via petrochemical synthesis alone. We will direct our attention to some of these materials in the remainder of this chapter, and in the balance of this book. Meanwhile, rubber, one of the first biotechnology derived polymers, remains a multibillion dollar business.

POLYSACCHARIDES: MATERIALS AND APPLICATIONS

Cellulosic derivatives, including CMC, various ethers, and additional variations, have long been the mainstay for biotechnological polymers, and are still widely used in the cosmetic, food, medical and drug release industries.[8] Cationic cellulosic derivatives, such as the Celquat materials from National Starch, have found application in cosmetic lotions and creams and hair care products, including shampoos and conditioners.[9] The methyl cellulose ether Methocel (Dow) is used in water-based paints, pigment flocculation, ceramic glazes, tanning, thickening, and as a food additive. The corresponding ethyl ether (Ethocel, Dow) has limited water solubility and is used in automotive parts and household articles. Sodium carboxymethylcellulose (CMC) is commonly used to prevent soil redeposition in synthetic detergent formulations. Other uses for CMC include: a binder, thickener, suspending agent, textile sizing, paper and paperboard coating, food additive, paints, cosmetics and pharmaceuticals.

Numerous other polysaccharides, such as konjac and carrageenan, find multipurpose applications in areas such as cosmetic, food and medicine. Konjac is used medically as a barrier film to protect wounds and in various controlled release systems. Carrageenan, a seaweed derivative, finds use in hand lotions, shaving creams, shampoos, dentifrices and controlled release.[10] Starch, aside from it use as a foodstuff, finds applications in cosmetics (as a thickener), pharmaceuticals (as a binder, filler, thickener or disintegrant), a binder for pigments on paper or textiles, and in miscellaneous places needing an inexpensive thickener. A recent publication describes simple laboratory procedures that can be used to make biodegradable, plastic films from starch.[11]

The treatment of abnormal skin conditions, long a mainstay of the cosmetic industry, is being pursued via numerous medical or pharmaceutical agents. Polysaccharides find utility in this area since several exhibit emollient behavior. In the last few years, certain retinoic acid derivatives have appeared on the market as presumed inter-active cosmetics, which are claimed to reduce wrinkles. Even though retinoic acid is fairly low molecular weight, Shiseido, a Japanese company, is marketing the sodium salt of hyaluronic acid, a natural polysaccharide, as a skin treating cosmetic.[12] Part of this cosmetic suitability for hyaluronic acid probably arises from its hygroscopic nature, which enables it to impart moisture to the skin. Hyaluronic acid also possess good skin adhesive characteristics, which could aid in cosmetic usefulness.

Hyaluronic acid exists naturally in the connective tissue of most vertebrates and can be isolated from scrap materials such as rooster combs. Its uses include the treatment of arthritis and eye surgery. In both cases, this polysaccharide achieves its special functions via a highly viscous solution. In addition, hyaluronic acid has been investigated for synovial fluid replacement. Hyaluronic acid has been utilized as a polymeric matrix in controlled release systems, where it behaves like a hydrogel.[13,14]

Chitin, a animal-derived polysaccharide closely related to cellulose, has a wide variety of biomedical, agricultural and cosmetic uses.[14,15,16] Found mainly in insect and crustacean shells, chitin is the animal equivalent of cellulose. Since chitin itself is insoluble in most solvents, most research centers on the deacetylated version, chitosan, which is now finding application in the textile industry, waste water treatment and medicine.[17] While neither material is likely to be made *synthetically* on a commercial scale, both polymers are derived from formerly useless waste materials. Several chitin or chitosan derived materials are currently marketed in Japan in various hair and skin care formulations.

Medical uses of chitin derivatives include potential replacement blood vessels, which possibly arises from chitin's similarity to heparin. Another derivative, carboxymethylchitin, has been used to prepare vesicles which function as drug carriers or possible artificial red blood cells.[18] Chitin derivatives also are being studied as artificial skin replacements (wound dressings).[19,20]

Heparin, a polysaccharide which prevents blood clotting, finds extensive biomedical usage as a blood additive, and has recently been reviewed comprehensively.[21] Heparin continues as the subject of much research, including blood substitute applications.[22] Numerous papers have appeared on heparin-like materials, called heparinoids, which can be synthesized from materials such as natural or synthetic rubber.[23,24]

Some recent studies have shown fungus derived polysaccharides possess anti-tumor properties.[25] Independent, recent research suggests that the polysaccharide curdlan sulfate has antiviral activity, including potential anti-AIDS activity.[26] Biologically active polysaccharides have been reviewed.[27] Several recent reports have shown that 5-fluorouracil can be attached to various polysaccharides, such as dextran, xylan and chitosan, but these systems did not exhibit zero-order release of the anti-cancer drug.[28]

POLYPEPTIDES: MATERIALS AND APPLICATIONS

Polypeptides have been used less as biotechnological polymers for several reasons. First, polypeptides tend to degrade more readily than desired for most applications, usually with rather unpleasant odors. Second, these polymers generally are obtained from animal sources which makes them somewhat more expensive than polysaccharides. Third, and most problematical, natural polypeptides are normally far more complicated in

structure than ordinary polysaccharides, which usually have a single repeat unit. (Note, however, that some polysaccharides are crosslinked via several different sites.) Polypeptides seldom exist as a single repeat unit material, although there are several important exceptions, but polypeptides are essentially intricate copolymers of several amino acids.

Nonetheless, several polypeptides have achieved fairly wide utility. The most common application is probably animal-based glues. Recently the structure of the polymer used by mussels to adhere to boat hulls has been determined,[29] and the polymer synthesized. It seems to be an excellent biotechnology-derived adhesive.[30,31] In 1983, J. H. Waite (U. Delaware) discovered that the protein secreted by the phenol gland of the blue sea mussel *Mytilus edulis* consisted of some closely related hexapeptide and decapeptide sequences, with a combined molecular weight of about 130,000.[32,33] This protein is transformed into the adhesive by enzymatic oxidation, anchoring the mussel to surfaces in an aqueous habitat.[34] The adhesive protein of the mussel *Mytilus edulis* protein consists mainly of the repeating decapeptide sequence Ala-Lys-Pro-Ser-Tyr-Hyp-Hyp-Thr-Tyr-Lys. Recombinant DNA techniques have been used to formulate and then clone a gene encoding this polydecapeptide analog protein, and this protein was successfully expressed in *Escherichia coli*. It showed an apparent molecular weight of 25,000.[35] The protein was also synthesized via a two step chemical process, forming first the decapeptide sequence in an Applied Biosystems model 430A automated synthesizer employing modified Merrifield procedures, and subsequently polymerizing the decapeptide to form the protein. The molecular weight ranges from 35,000 to 65,000. This mussel adhesive protein is also appealing because it combines the properties of nontoxicity, durability and biocompatiblity, and could find use in the medical sphere as wound closures, bone and/or dental repairs and tissue bonding.[36]

Gelatin, a polypeptide, finds extensive use in microcapsules for use in drug delivery and in the encapsulation of various water-insoluble oils used in cosmetics. Gelatin systems are normally crosslinked to nullify the water solubility of the polymer. Although it's another old polypeptide, dozens of papers appear annually on potential medical uses of collagen, including skin and nerve regeneration[37,38,39] and artificial blood vessels.[40] (The skin and nerve regeneration normally utilizes a matrix of collagen with chondroitin sulfate.) Collagen has been used to immobilize percutaneous implants,[41] and has been used in controlled release applications.[42,43] For many decades, collagen has been used cosmetically for treating the scars from small pox and correcting facial defects.

Polyelastin, essentially a synthetic polypeptide patterned after natural elastin, is being examined in both the medical and controlled release areas.[44,45] Although relatively new, and of synthetic origin, polyelastin could probably find utility in cosmetics, if the cost is not too great.[46] Other important polypeptide uses include biodegradable drug delivery systems,[42] drug carriers,[47] and pharmaceutical agents.[48] Enzymes, of course, are polypeptides with very high catalytic activity, and several groups are synthesizing modified proteins with "artificial" catalytic activity.[49] Recently, catalytic antibodies, also polypeptides, have been

developed.[50] In addition, the concept of totally artificial enzymes has been pursued vigorously by several groups, and has been reviewed.[51] Immobilized enzyme research also continues.[52] The enkephalins and endorphins, a special type of polypeptide found in the brain, are powerful analgesics, and many modifications have been made on these natural polymers which may ultimately be marketed as drugs.[53,54,55] Many other polypeptides have been studied as potential pharmaceutical agents, and this topic has been reviewed recently.[48]

SPIDER SILKS

Spider silk, a polypeptide, has received much attention as a novel biotechnological polymer because it is five times stronger than steel, can be stretched 130%, is water resistant and fairly chemically inert. Spider silk is not a single entity; some spiders can spin at least seven chemically-different kinds of silk. Some potential applications that are being considered include fibers for wound repair, bullet-proof vests, parachutes, catch-cords for aircraft carriers, machine-washable silk-garments, and artificial ligaments and tendons, but not all types of spider silk can work for all these uses. For example, elasticity may be useful in catching flies, but it is scarcely desirable in a bullet-proof vest where both the missile and vest could penetrate through the chest and inflict harm on the wearer, even if the bullet were prevented from lodging in the body. Likewise, harvesting spiders for the garment industry poses several problems, including the fact that the total output of several thousand spiders would be needed in order to fashion one small garment. Spiders cannot, unfortunately, be raised on farms like silkworms.[56,57]

Protein Polymer Technologies (Joseph Capello, San Diego) introduced ProNectin F in early 1991. This polypeptide, based on eight amino acids arranged in a precise sequence, is claimed to form transparent coatings on other materials, such as petri dishes, and can be used as a base on which to grow cells. On fabrics, ProNectin F is claimed to impart a silk-like luster and texture, and to improve breathability. This company is expected to introduce as second polypeptide, BetaSilk, in 1992; this is claimed to improve other textiles by making them softer and more breathable.[58,59] Monsanto, largely under the direction of Stephen J. Brewer, is also examining spider silks, and related polymers.

Research at the University of Wyoming (Randy Lewis) and the U. S. Army's Natick Research Center (David L. Kaplan) are studying ways to clone spider silk into various bacteria, such as E. coli, and yeasts. When this is done, however, the material is retrieved as a bulk polymer, instead of a fiber.[58] The spinning of this polymer into silk-like fibers has yet to be solved in a manner suitable for mass production.[59] Research on this problem is underway at the University of Washington (Chris Viney), the University of Wyoming (Randy Lewis), and elsewhere. Viney identifies three phases in the production of spider silk: (1) production of a disorganized polypeptide in the spider's abdominal glands, (2) a liquid-crystal phase in which a crystallized portion of the molecules become aligned in the same direction, and (3) the spun fiber wherein these

polymeric crystals are embedded within a disordered array of protein chains.

POLYESTERS: MATERIALS AND APPLICATIONS

As noted earlier, shellac is a natural polyester which is still used in the coatings industry. The dominant biotechnological-derived polyesters are, however, the poly-ß-hydroxyalkanoates, which are a family of natural polyesters that usually exist as copolymers with homologous alkane side chains. Biologically, these polymers constitute the food reserve of the bacterial world.[60,61] The commercial version of PHA, available from ICI, is a random copolymer with butyrate and valerate repeating units. In a sense, these materials can be considered biotechnology mimics of polyolefins.[62,63] Poly-ß-hydroxybutyrate (PHB) is the first member of the series. The basic repeat unit structure is shown below, and a whole family of copolyesters exists.[64] These BHA copolymers normally biodegrade readily.[65,66]

$$[-O-CH-CH_2-C-]$$
with R and O substituents
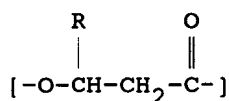
$$R = CH_3, C_2H_5, CH_5H_{11}, C_6H_{13}, etc.$$

These copolymers are thermoplastics. Depending on the specific composition, the T_g can range from +10 to -40°C as the side chain length varies from C_1 to C_6. Likewise, the T_m ranges between 184 and approximately 50°C, while the density varies between 1.24 and 1.04 g/cc. This thermoplastic changes from a material of moderately high crystalline content (approximately 60%) into an elastomer with only about 25% crystallinity as the side chain length increases.[64,67,68,69,70]

Commercial exploitation of PHB was achieved by Imperial Chemical Industries (ICI) via a fermentation route by adding varying amounts of propionic acid to a pure carbohydrate substrate. This resulted in the copolyesters which contain only butyrate (HB) and valerate (HV) comonomer units.[71] In this biosynthesis of PHB/HV, enzymes form a micelle-like structure around the individual granules, possibly furnishing some control on the size of the specific granules.[72] In some ways, the ICI isolation process is similar to wood pulping since the granules persist while the cell wall, proteins, nucleic acids, etc. are removed by mechanical and enzymatic processes.[71,73] PHA copolymers can also be synthesized via a ring-opening polymerization of the appropriate lactones.[74]

One of the major obstacles to widespread usage of the PHAs is cost. Biopol, the ICI polymer from its Billingham England plant which can produce about 5×10^5 pounds annually via bacterial fermentation, costs approximately $15/lb. ICI expects that improved manufacturing techniques will reduce this cost to around $3/lb, but this is still significantly higher than the typical $0.60/lb for petroleum-based polymers. Although the PHB polymer is more expensive, Wella Corp. does use it to manufacture

bottles for use in "green" shampoos, mostly in Germany.[75] Further cost reductions will likely require new methods for producing the polymer, and several groups are attacking this problem worldwide.

Using plasmid technology, Donald Dennis (James Madison U.) cloned the genes for PHB synthesis into *E. coli*, which then produced the polymer.[76] More recently, Chris Somerville, and coworkers at Michigan State U. and James Madison U., have inserted the genes from the bacterium *Alcaligenes eutrophus*, which promote the formation of polyhydroxybutyrate, into several kinds of higher plants. These plants included *Arabidopsis thaliana*, a mustard-like weed.[77] Studies are continuing on other plants, such as potatoes and corn. They estimate that the cost of the polymer could decrease to about 10% of the current level once these procedures become commercial. At about $1.50/lb, PHB could become a robust ingredient in the polymer market.

The polylactides (PLAs), and the related polyglycolides, are another class of biotechnological polyesters which have been around for many years. They have been used in medical applications for many years as resorbable bone plates,[78,79] and controlled release systems for drugs.[80,81] The PLA polymer, which was discovered in the 1930s by W. H. Carothers, is totally biodegradable, but is also expensive to manufacture. A recently developed process at the Argonne National Laboratories, called the BioLac process, is claimed to reduce this cost significantly, permitting application in such diverse areas as compost bags, agricultural controlled release devices, and seed or fertilizer coatings. This new process enzymatically converts food wastes, such as cheese whey or potato scrap, into glucose, which is then transformed enzymatically into lactic acid. The lactic acid is converted into the polymer. In addition to producing this polymer more cheaply, the BioLac process can also dispose of more than five million tons of potato waste and about the same amount of waste whey.[82,83]

NUCLEIC ACID ANALOGS

While they appear unlikely to achieve the volume of several other biotechnological polymers, nucleic acid analogs also have potential commercial applications, mostly in the medical and agricultural fields. Numerous research groups have studied synthetic analogs of nucleic acids, including vinyl or (meth)acryloyl derivatives of the nucleic bases,[84,85,86,87,88] and poly(alkylene phosphates) and related species.[89,90,91,92] Some nucleic base polymers can act as activators for the immune system.[93]

Our research has centered partially on analogous nucleic acids in which the antitumor drugs 5-fluorouracil or 6-methylthiopurine units replaced the typical nucleic bases. We prepared synthetic methacrylate analogs of these pseudo-nucleic bases and then studied the polymerization, copolymerization, and the controlled release of the antitumor agents. These copolymers showed a potentially-valuable, zero-order release of the antitumor agent.[94,95,96] Such systems could provide improved chemotherapy regimens, with long-term effectiveness combined with reduced side

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