Technical Overview of Biopolymer Field

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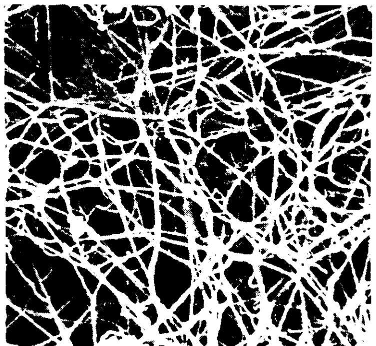
Bcopolymers can be produced through a variety of mechanisms. They can be derived from microbial systems, extracted from higher organisms such as plants, or synthesized chemically from basic biological building blocks. A wide range of emerging applications rely on all three of these production techniques. Biopolymers are being developed for use as medical materials, packaging, cosmetics, food additives, clothing fabrics, water treatment chemicals, industrial plastics, absorbents, biosensors, and even data storage elements. This chapter identifies the possible commercial applications and describes the various methods of production of some of the more promising materials. Table 2-1 provides a partial list of the biopolymers now in use. ’

SELECTED POLYMERS PRODUCED BY MICROBIAL SYSTEMS

In recent years, considerable attention has been given to biopolymers produced by microbes. It is on the microbial level where the tools of genetic engineering can be most readily applied. A number of novel materials are now being developed or introduced into the market. In the following sections, three



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[1] A comprehensive treatment of biopolymer compounds is beyond the scope of this Background Paper. The categories of biopolymers described here are designed to illustrate the diverse physical characteristics and the broad application range of these materials. For a more detailed discussion of biopolymers, see David Byrom (cd.), *Biomaterials: Novel Materials from Biological Sources* (New York, NY: Stockton Press, 199 1); Roger Rowell, Tor Schultz, and Ramani Narayan (eds.), *Emerging Technologies for Materials and Chemicals from Biomass* (Washington, DC: American Chemical Society, 1992); David Kaplan et al., ‘‘Nahmally Occurring Biodegradable Polymers,’ G. Swift and R. Narayan (eds.), *Polymer Systems—Synthesis and Utility* (New York, NY: Hanser Publishing, forthcoming 1994).

Table 2-l—A Snapshot of the Biopolymer Family

- Polyesters
  - Polyhydroxyalkanoates
  - Polylactic acid
- Proteins
  - Silks
  - Collagen/gelatin
  - Elastin
  - Resilin
  - Adhesives
  - Polyamino acids
  - Soy, zein, wheat gluten, casein,
  - Serum albumin
- Polysaccharides (bacterial)
  - Xanthan
  - Dextran
  - Gellan
  - Levan
  - Curdlan
  - Polygalactosamine
  - Cellulose (bacterial)
- Polysaccharides (fungal)
  - Pullulan
  - Elsinan
  - Yeast glucans
- Polysaccharides (plant/algal)
  - Starch (amylose/amylopectin)
  - Cellulose
  - Agar
  - Alginate
  - Carrageenan
  - Pectin
  - Konjac
  - Various gums (e.g., guar)
- Polysaccharides (animal)
  - Chitin/chitosan
  - Hyaluronic acid
- Lipids/surfactants
  - Acetoglycerides, waxes, surfactants
  - Emulsan
- Polyphenols
  - Lignin
  - Tannin
  - Humic acid
- Specialty polymers
  - Shellac
  - Poly-gamma-glutamic acid
  - Natural rubber
  - Synthetic polymers from natural fats and oils (e.g., nylon from castor oil)

SOURCE David L Kaplan et al , “Naturally Occurring Biodegradable Polymers,” G Swift and R Narayan (eds), *Polymer .Systems-Synthesis and Utility (New* York, NY Hanser Publishing, forthcoming 1994)

different classes of microbially derived biopolymers are profiled: polyesters, proteins, and polysaccharides.

Microbial Polyesters: Polyhydroxyalkanoates

Much of the current interest in biopolymers stems from the growing concern about the environmental impacts of synthetically produced materials. In particular, the highly publicized disposal problem of traditional oil-based thermoplastics has promoted the search for biodegradable alternatives (about 17 billion pounds of thermoplastic packaging material was produced in the United States in 1991). Apart from the agriculturally derived biopolymers (e.g., starch) being investigated for their biodegradable properties, there is a class of natural thermoplastic materials that is drawing much attention. Polyhydroxyalkanoates (PHAs) area family of microbial energy reserve materials that accumulate as granules within the cytoplasm of cells. They are genuine polyester thermoplastics with properties similar to oil-derived polymers (melting temperatures between 50 to 180”C). Their mechanical characteristics can be tailored to resemble elastic rubber or hard crystalline plastic.[3] The prototype of this family, polyhydroxybutyrate (PHB), was frost discovered in 1927 at the Pasteur Institute in Paris. Commercialization of PHB was first attempted by W.R. Grace Co. in the 1950s. More recently, a British company, Zeneca Bio Products (formerly ICI Bio Products), initiated commercial production of a series of PHA copolymers under the trade name BIOPOL™. Several companies and government research organizations, particularly in Europe and Japan, have active research and development (R&D) programs focusing on these materials.

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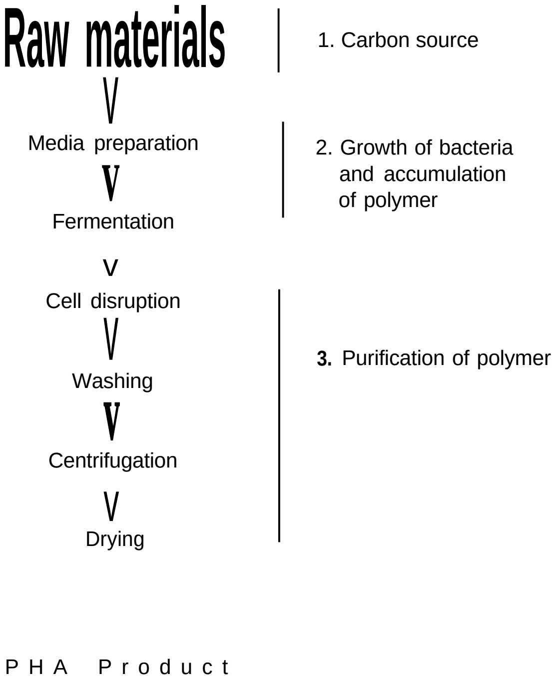
[2] Thermoplastics are polymers that will repeatedly soften when heated and harden when cooled.

[3] y. Doi, ‘‘~crobiat synthesis and Properties of Polyhydroxyalkanoates, *Materials Research Bulletin,* vol. 17, No. 11, November 1992, pp. 39-42.

Production of PHAs is carried out through fermentation. The general process is illustrated in figure 2-1. In their final stages of preparation, they can be processed by standard extrusion and molding techniques. Careful control of the carbon sources (starting materials) and the choice of production organism enables the production of an entire family of PHA copolymers with different properties. The PHB homopolymer is produced by a variety of bacteria that use it as a source of carbon and energy. The homopolymer is a brittle material that is difficult to use and is thermally unstable. However, by combining polyhydroxyvalerate (PHV) with PHB, a nonbrittle copolymer—polyhydroxybutyrate-polyhydroxyvalerate (PHBV)--can be created.[4] Other PHA copolymers have also been produced.[5] Currently, Zeneca's production of PHBV (BIOPOL) uses the bacterium *Alcaligenes eutrophus,* which occurs widely in soil and water. PHBV is formed when the bacterium is fed a precise combination of glucose and propionic acid.[6] It has properties similar to polypropylene and polyethylene, including excellent flexibility and toughness. The discovery and development of PHBV and other PHA copolymers have proven to be a major step forward in expanding the potential industrial utility of the PHAs.

Figure 2-I—PHA Polymer Production Process



SOURCE: BioInformation Associates, Boston, MA.

PHAs biodegrade in microbially active environments. Since PHAs function as an intracellular energy and carbon source, bacteria can degrade PHAs and use them as reserve materials. Microorganisms attack PHBV by secreting enzymes (depolymerases) that break down the polymer into its basic hydroxybutyrate (HB) and hydroxyvalerate (HV) constituents. The HB and HV fragments are then consumed by the cells to sustain growth. Under aerobic conditions, the final biodegradation products are water and carbon dioxide; under anaerobic conditions, methane is produced as well. The degradation of PHBV can be quite rapid in biologically active systems (see figure 2-2).[7] A range of soil microor-

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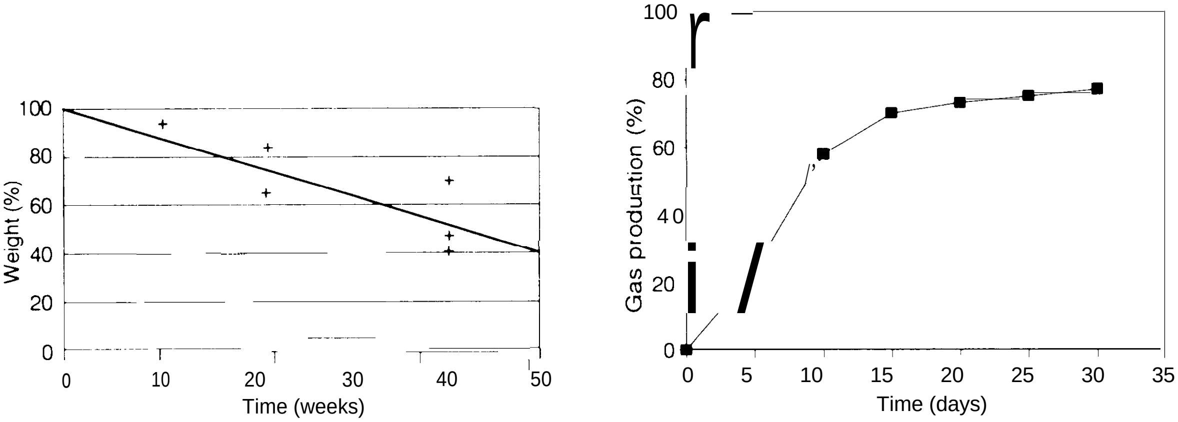
[4] William D. Luzier, ‘ ‘Materials Derived from Biomass/Biodegradable Materials,’ National Academy of Sciences, vol. 89, February 1992, pp. 839-842.

[5] For example, researchers at the Tokyo Institute of Technology have produced a versatile PHA copolymer of 3-hydroxybutyrate and 4-hydroxybutyrate. This material has been extracted and fermented from the bacterium *Alcaligenes eutrophus* under starvation conditions. By altering the ratio of the copolymers, the elasticity and strength of the material can be varied (Doi, op. cit., footnote 3).

[6] Glucose is derived from agricultural feedstocks such as sugar beets and cereal crops, whereas propionic acid can be produced from petroleum derivatives or by fermentation of wood pulp waste.

[7] When 50 bottles made of PHBV material were inserted into a compost heap (6 cubic meters of organic waste) for a period of 15 weeks, at temperatures between 60 and 70”C, only about 20 percent (by weight) of the PHBV material remained. It is important to note, however, that PHBV and other biopolymers will not degrade in sanitary landfills, because they are essentially biologically inactive systems. See Petra Püchner and Wolf-Rüdiger Müller, “Aspects on Biodegradation of PHA,'' H.G. Schlegel and A. Steinbüchl (eds.), *Proceedings International Symposium on Bacterial Polyhydroxyalkanoates 1992* (Göttingen: Goltze-Druck, 1993).

Figure 2-2—Biodegradation of PHBV Polymers



Degradation of PHBV landfill simulation

Anaerobic degradation of PHBV 27% copolymer

PHBV can degrade relatively quickly in biologically active systems. in a simulated landfill environment with an elevated moisture content, PHBV showed a 40-percent weight loss In 40 weeks (left). Under anaerobic sewage conditions, where biodegradability is measured by gas production, the PHBV polymer decomposed nearly 80 percent in 30 days (right). Other data indicates that PHBV readily degrades under aerobic conditions.

SOURCES: Petra Pochner and Wolf-Rudiger Muller, "Aspects on Biodegradation of PHA," H.G. Schlegel and A. Steinbüchl (eds.), *Proceedings International Symposium on Bacterial Polyhydroxyalkanoates 7992* (Göttingen, Goltze-Druck: 1993); William D. Luzier, "Materials Derived from Biomass/Biodegradable Materials," *Proceedings National Academy of Sciences, vol. 89,* February 1992, pp. 839-842.

ganisms, both bacterial and fungal, can utilize PHAs as a source of carbon and energy.

CURRENT AND POTENTIAL APPLICATIONS

PHAs have many possible uses. The inherent biocompatibility[8] of these bacterial materials suggests several medical applications: controlled drug release, surgical sutures, bone plates, and wound care. PHAs could also be used as structural materials in personal hygiene products and packaging applications. At present, higher-volume commodity plastic applications are limited because of economic constraints. PHBV, for example, costs $8 to 9 per pound. However, costs have fallen from about $800 per pound in 1980, and it is believed that the price of PHBV can be brought to around $4 per pound by 1995. (Petroleum-based polyethylene and polypropylene polymers cost about 50 cents per pound.) PHBV is now being used in a variety of molding applications in the personal care sector (e.g., biodegradable cosmetic containers-a market where the cost of the container is almost negligible in relation to the cost of the contents). In addition, Zeneca is in the process of commercializing films and paper coatings from BIOPOL resin. Mitsubishi Kasei (Japan) is actively involved in the development of PHAs for use as a biodegradable replacement of monofilament fishing nets. Because of the desirable environmental characteristics of PHAs, the number of such niche markets is likely to multiply.

Over the past 5 years, there has been a substantial increase in the number of publications dealing with the biosynthesis, fermentation, and characterization of the PHA family of biopolymers. It soon may be possible for these polymers to compete as specialty plastic products. The ability to genetically engineer the different spe-

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[8] The final degradation product of one type of PHB is a normal constituent of human blood.

cies of bacteria used to produce PHAs (e.g., by modifying the enzymes inside the bacteria) could result in the creation of highly customized polymers. Researchers have made significant progress in unraveling the biosynthetic pathways involved in the production of PHAs. The genes encoding the enzymes involved in PHA production have been isolated and cloned, and thus scientists can now tailor the biosynthesis process to produce polymers with different properties. Over the long term, there is the possibility that these materials can be made economically in plant species.[9]

Recently, PHB was successfully synthesized by using a genetically engineered experimental plant *(Arabidopsis thaliana).*[10] Researchers are now exploring how to produce PHAs by modifying the enzyme machinery of corn or potatoes.[11] Although significant technical challenges remain, the PHA family could potentially become a major agricultural commodity, either as a fermentation product using raw materials (e.g., glucose) from the starch industry or, in the longer term, as a new crop.

Recombinant Protein Polymers

Proteins are polymers composed of amino acids. The specific amino acids used and the sequence of amino acids in a protein polymer chain are determined by the corresponding DNA template. Many proteins are of commercial interest because of their catalytic (enzymatic) or pharmaceutical properties. However, nature has also provided a vast array of proteins whose principal function is to form structural materials in living organisms. Some of the more familiar protein materials include wool, leather, silk, and gelatin (jello is a simple, modified form of the protein collagen[12]). Although many of these structural proteins have been used throughout history, the advances in recombinant DNA technology have presented new approaches and opportunities for the design and synthesis of protein materials. In addition, many traditional proteins prepared by the extraction of animal (e.g., collagen) or plant (e.g., soy or zein from corn) tissue are being chemically or physically modified for


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Coatings based on corn protein (zein) have good moisture and grease barrier properties and are being used to replace polyethylene and wax-coated paper and paperboard.

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[9] The genetic manipulation of bacteria could also alter the economics of PHA production. However, the manufacturing costs for PHAs are determined primarily by the purification steps rather than the bacterial production steps (see figure 2-l).

[10] Although early work in this area is encouraging, achieving controlled gene expression of PHB in plants is a formidable undertaking. Inserting the genes that encode the PHB-producing enzymes is relatively straightforward. However, regulating the PHB enzymes and the existing plant enzymes is a more difficult challenge. See Yves Poirier, Douglas Dennis, Karen Klomparens, and Chris Somerville, ‘ ‘Polyhydroxybutyrate, a Biodegradable Thermoplastic, Produced in Transgenic Plants,” *Science,* vol. 256, Apr. 24, 1992, pp. 520-523.

[11] Robert Pool, ‘‘In Search of the Plastic Potato, ” *Science,* vol. 245, Sept. 15, 1989, pp. 1187-1189.

[12] Collagen is a fibrous protein that is the principal component of animal connective tissue. It is the most abundant of all proteins found in mammals, typically accounting for more than 30 percent of body protein. The arrangement of collagen fibers depends on the nature of the tissue. For example, in tendons, fibers are arranged parallel to one another to give a structure with the tensile strength of a light steel wire. In skin, where strength and flexibility are required, collagen fibers are randomly oriented and woven together like felt.

Table 2-2—Repeat Units Found in Protein Materials

Protein	Source	Amino acid repeat unit[a]
Silk	Silk worm	GAGAGS
Collagen	Mammals	GPP[b]
Adhesin	Mussel	AKPSYPPTYK
Elastin	Pig	VPGVG
Synthetic	Chemically synthesized genes	Various

[a] There are 20 different amino acids, designated here in short form: A = alanine; G = glycine; K = lysine; P = proline; S = Serine; T = threonine; V = valine; and Y = tyrosine. For a full list of amino acid symbols, see Albert Lehninger, *Biochemistry* (New York, NY: Worth Publishers, 1975), p. 72.

[b] Often one of the prolines in collagen is hydroxyproline.

SOURCES: BioInformation Associates, Boston, MA; Office of Technology Assessment.

new applications in the biotechnology and food industries.[13] The discussion here focuses on protein polymers that are being developed by using the methods of recombinant biotechnology.

PRODUCTION OF RECOMBINANT PROTEIN POLYMERS

The extraordinary functional diversity of natural proteins underscores the potential advantages associated with harnessing the genetic code. In theory, proteins can be designed to have virtually any structure, and thus specific physical and chemical properties. The fact that one-dimensional genetic sequences can specify proteins having complex three-dimensional structures over distances of hundreds of nanometers reveals the power of nature's material synthesis processes. Current chemical synthesis techniques are essentially limited to creating polymers in one dimension only,[14] with lengths of less than 10 nanometers.[15]

A number of proteins that form important structural materials in various organisms have been studied extensively. The fibrous proteins such as collagen and silk have been the subject of considerable attention. More specialized proteins, such as the adhesive material that bonds the sea mussel to the ocean bed and proteins that contribute to the formation of 'ceramic-like' materials (e.g., oyster shells and teeth), are also being actively studied. A common feature of many of these proteins is the presence of repeated amino acid sequences in the polymer product (see table 2-2). These proteins, or specific regions of these proteins, have structural features similar to block copolymers (see figure 1-1).

Some of the structural proteins have been chemically synthesized. In this approach, specific peptides (sequences of amino acids) are created and then linked together to form a polypeptide (the protein polymer). However, chemical synthesis of proteins can be quite expensive[16] and

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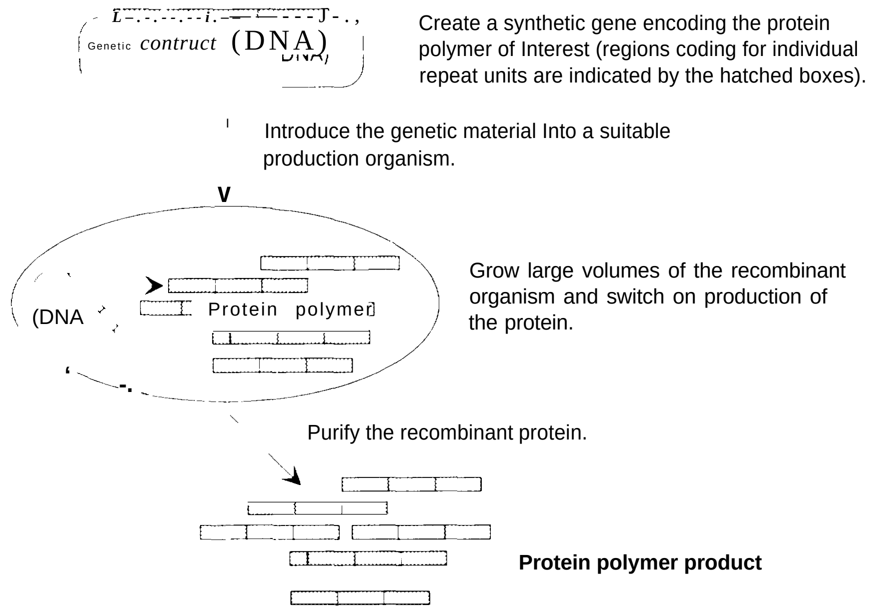
[13] Soy and corn proteins are used primarily as coatings for paper and paperboard. Soy paper coatings impart a smoother surface and improve surface appearance. Zein has excellent grease and moisture barrier properties. Soy proteins are also being used as structuring agents in water-based inks, because they are regarded as environmentally preferable to solvent-based inks. See Thomas L. Krinski, "Emerging Polymeric Materials Based on Soy Protein,'' Rowell et al. (eds.), op. cit., footnote 1.

[14] There have been some advances recently in the synthesis of two-dimensional synthetic polymers. However, this work is still somewhat preliminary. See *Scientific American,* "Flat Chemistry," April 1993, p, 26.

[15] See Joseph Cappello, ''Genetic Production of Synthetic Protein Polymers," Materials *Research Bulletin,* October 1992, pp. 4S-53.

[16] In a few cases, though, some firms have been able to develop relatively inexpensive approaches for synthesizing complex polypeptides. For example, Monsanto developed commercial chemical methods for making somatotropin, a complicated polypeptide, for about $15 per pound.

Figure 2-3—Production of Recombinant Protein Polymers



Create a synthetic gene encoding the protein polymer of Interest (regions coding for individual repeat units are indicated by the hatched boxes).

Introduce the genetic material Into a suitable production organism.

Grow large volumes of the recombinant organism and switch on production of the protein.

Purify the recombinant protein.

Protein polymer product

SOURCE: BioInformation Associates, Boston, MA.

typically does not yield products with sufficient physical and chemical uniformity.

In contrast, the recombinant DNA approach allows for the production of protein polymers that have high purity, specific molecular weights, and excellent lot-to-lot uniformity. This is possible because the exact sequence of amino acids in the polymer and the desired molecular weight are specified by the DNA sequence of the gene. In the long term, recombinant DNA technology could become an important strategy for producing high-value, ‘‘knowledge-intensive’ materials that are custom-designed for specific applications. The steps involved in producing recombinant proteins are illustrated in figure 2-3, Currently, all of the proteins shown in table 2-2 are being produced or investigated by using recombinant DNA techniques. The fact that it is now possible to chemically synthesize genes encoding any repeat unit suggests almost limitless possibilities for creating novel protein polymers with unique physical and functional properties. Although researchers are addressing major technical problems such as genetic instability, toxicity in foreign hosts, and metabolic incompatibility, considerable progress in genetically engineered protein synthesis has occurred in recent years.[17] Initial work in the synthesis of artificial genes has led to the creation of proteins that could be used as coatings and adhesives, membrane separators, and medical and optical materials.[18]

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[17] Because foreign DNA imposes a metabolic burden on host cells, in some cases these DNA segments can be destroyed. In particular, the highly repetitive gene sequences that are necessary to create artificial protein polymers are frequently unstable in microorganisms. In addition, novel proteins that are encoded by synthetic genes maybe toxic to the host cells, thus causing cell death before the polymer can be accumulated in useful quantities. See David A. Tirrell et al., ‘ ‘Genetic Engineering of Polymeric Materials, ’ *Materials Research Bulletin,* July 1991, pp. 23-28; see also Joseph Cappello, op. cit., footnote 15.

[18] See *R&D Magazine,* ‘‘Bioderived Materials, ” June 1990, pp. 58-64.

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The marine mussel, Mytilus edulis, is shown suspended in water by threads attached with adhesive to a glass plate. Biotechnology research on adhesives produced by rnarine organisms is leading to new adhesives that can be used for many applications such as surgery and undersea structures.

CURRENT AND POTENTIAL APPLICATIONS

More than 25 genetically tailored proteins have been synthesized in microorganisms. Many of these materials are being transformed into films, gels, and fibers. One of the first genetically engineered protein polymers to be introduced commercially, ProNectin F™[19] was designed to serve as an adhesive coating in cell culture vessels. The polymer was customized to have two distinct peptide blocks: one block possesses the strong structural attributes of silk; the other has the cell-binding properties of the human protein fibronectin.[20] The peptide blocks were chosen after analyzing which particular structures could provide the desired physical, chemical, and biological properties. ProNectin F has demonstrated excellent adhesion to plastic surfaces such as polystyrene and thus can be used to attach mammalian cells to synthetic substrates.[21]

A similar application of recombinant DNA technology has led to the development of a bioadhesive based on a protein from the sea mussel Mytilus *edulus.*[22] Researchers have genetically modified yeast cells to produce the basic mussel protein. An enzyme-catalyzed process (the enzyme-a tyrosinase-modifies the tyrosine amino acids in the protein) was also developed to convert this recombinant protein into a true adhesive.[23] This polymer could be used as a marine coating, as a wetting agent for fibers in composite materials, or as a dental or surgical adhesive.[24] For example, it might be employed as a sealant during eye surgery.[25]

A number of other protein polymers are being investigated by using biotechnology methods. One intriguing new material is a polypeptide based on the natural protein elastin found in cows

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[19] The protein is produced by Protein Polymer Technologies, Inc., San Diego, CA.

[20] Cappello, op. cit., footnote 15.

[21] With certain types of cells, ProNectin F displayed adhesive characteristics superior to that of natural attachment proteins including fibronectin. Ibid.

[22] Enzon Corp. (formerly Genex Corp.) developed the genetically designed adhesive under the name AdheraCell. See *Chemical and Engineering News,* "Biotechnology Providing Springboard to New Functional Materials," July 16, 1990, pp. 26-32.

[23] This enzyme-catalyzed procedure is known as "post-translational" modification. The sea mussel performs this modification after protein biosynthesis.

[24] *Chemical and Engineering* News, op. Cit., footnote 22.

[25] *R&D* Magazine, op. cit., footnote 18.

and pigs.[26] This rubberlike material responds to changes in temperature and is able to convert chemical energy into mechanical energy.[27] As a consequence, the material could be used as a replacement for ligament tissue, blood vessels, or any other tissues requiring the contractile properties of elastin.[28] Because the peptide is similar in composition to natural elastin, the polymer is not expected to elicit an allergic reaction or unfavorable immune system response.

Another interesting material is the light-sensitive protein bacteriorhodopsin (BR). BR is a retinal protein consisting of 248 amino acids. When subjected to photons (light) of varying energy intensity, BR undergoes reversible color changes. Through substitution of specific amino acids by genetic engineering, the photochemical properties of BR can be precisely modified, which means color changes can be carefully controlled. The unique properties of BR could lead to applications in optical data storage, image processing, light switching (computational processing), and holography. Some initial work indicates that the optical performance of BR is comparable to conventional materials such as liquid crystals.[29]

Recombinant biotechnology is also being used to modify materials that have been utilized for thousands of years. Silk has always been a material of great fascination. Spiders can process silk protein into a material that has a tensile strength 16 times greater than that of nylon[30] and a very high degree of elasticity.[31] Researchers have manipulated the genetic code to create silklike materials with a variety of elasticities.[32] Because of their exceptional tensile strength and elastic properties, these polymers could be used as fibers for reinforced plastics and other advanced composite materials. In the medical area, the polymers could potentially be used as wound dressings, artificial ligaments, and skin or as a biocompatible coating for prosthetic devices. However, the yields of genetically modified silk polymers from microorganisms have thus far been fairly low.[33]

Most protein polymer research is focused on high-technology applications, such as elastomers, adhesives, bioceramics, and electro-optical materials. To date, commercial applications have been limited to the use of genetically engineered adhesives for fixing mammalian cells to culture vessels. Because of extremely high production costs, these products will probably have limited

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[24] Elastin fibers are elastic, load-bearing protein polymers found in connective tissue such as ligaments. Another protein similar to elastin is resilin, a rubberlike polymer found in insects.

[27] See *Science,* “Heeding the Call of the Wild, ” vol. 253, Aug. 30, 1991, pp. %5-%8.

[28] *R&D* Magazine, op. cit., footnote 18.

[29] See N. Hampp, C. Brauchle, and D. Oesterhelt, “Mutated Bacteriorhodopsins: Competitive Materials for Optical Information Processing?” *Materials Research Bulletin,* vol. 17, No. 11, November 1992, pp. 56-60.

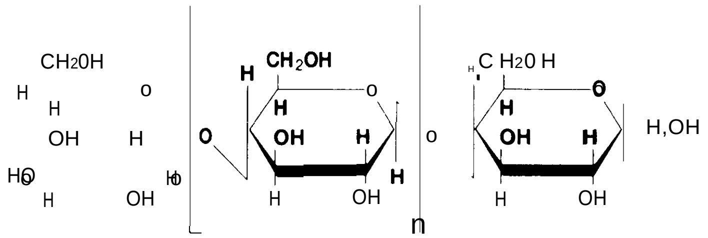
[30] Silkworm silk is about two to three times stronger than nylon.

[31] Spider dragline silk not only possesses great strength, but also has the ability to ‘‘supercontract.’’ Silk fiber will contract to less than 60 percent of its original length when wet, This results in a nearly thousandfold decrease in the elastic modulus and an enhanced ability to extend when necessary. This property allows the spider web to tighten each day when wetted with dew, while still maintaining its shape and tension. Although some human-made materials can sup-contract in organic solvents, no synthetic materials can supercontract-as spider silk does-in water alone. The web is constructed of several different silks, each of which is produced in a different spider gland. Some of the silk fibers contain a number of water-soluble compounds that keep the fibers wetted, allowing them to stretch and entangle prey that hit the web. See Randolf V. Lewis, “Spider Silk: The Unraveling of a Mystery, “ *Accounts of Chemical Research,* vol. 25, No. 9, 1992, pp. 392-398.

[32] Protein Polymer Technologies, Inc. has produced recombinant polymers based on silkworm silk. The company has developed an artificial silk gene that appears to be stable in host *Escherichia coli* cells. However, the stability of cloned silk genes in host systems still remains a significant problem *(R&D Magazine,* op. cit., footnote 18; *Chemical and Engineering News,* op. cit., footnote 22. Also see David L. Kaplan et al., “Biosynthesis and Processing of Silk Proteins, ’ *Materials Research Bulletin,* October 1992, pp. 41-47.

[33] Lewis, op. cit., footnote 31.

Figure 2-&The Structure of Cellulose



The cellulose molecule is composed of glucose units connected by B(1-4) bonds (see Figure 1 -3). Starch has an identical chemical composition to cellulose except for its connecting bonds-a(l -4). The different linkages in starch molecules endow them with a greater water volubility than cellulose. In humans, starch can be digested while cellulose cannot be digested.

SOURCE: Office of Technology Assessment, 1993.

success (e.g., experimental quantities of the genetically derived sea mussel adhesive were at one time selling for about $45 per milligram, or $20 million per pound). However, over the long term, genetic techniques may allow production to be scaled up significantly at reasonable cost. Once programmed with the proper genetic instructions, bacterial cells can work in parallel by the billions to produce polymer materials.[34] Although some biotechnology companies have been involved in protein polymer research for 10 years, most recombinant protein materials are still in early stages of development.

Nevertheless, this area of research is one of the most active and better funded in the biopolymer field. In addition to providing new materials, genetic engineering is now enabling scientists to study how biological systems transform proteins into final products. It is remarkable that living organisms are able to produce sophisticated materials under mild processing conditions (i.e., low temperature and pressure in water-based environments), without creating toxic byproducts. This is certainly not the case for a variety of human-made materials.[35] Spiders and silkworms, for example, can transform water-soluble protein droplets into globally aligned insoluble fibers.[36] The fibers are spun with very little energy consumption. Thus, protein polymer research could also lead to the development of radically new industrial processing methods that pose little threat to the environment.

The Microbial Polysaccharides

BACTERIAL CELLULOSE

Cellulose is the most abundant component of biomass and the basic feedstock of the paper and pulp industries. Traditionally extracted from plant tissue (trees, cotton, etc.), cellulose can also be produced by certain bacterial species by fermentation, yielding a very pure cellulose product with unique properties.

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[34] *Science*, op. cit., footnote 27.

3s Many advanced materials are synthesized at extremely high temperature and pressure, and require toxic substances at various stages of processing. See U.S. Congress, Office of Technology Assessment, *Advanced Materials by* Design, OTA-E-351 (Washington, DC: U.S. Government Printing Office, June 1988).

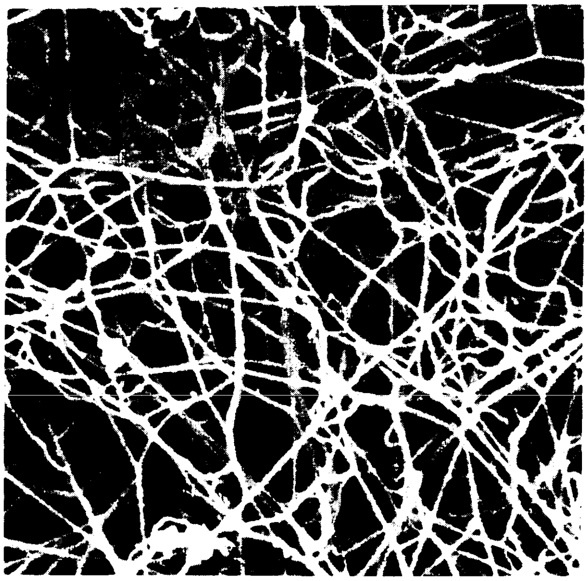
[36] Silk fibers are pulled from spiders, not forced out by pressure. The fibers are formed as they travel down a tubular duct kid@ from the gland to the exit valve. The key chemical and physical events that change the soluble proteins into solid fibers occur during this journey. As the protein molecules travel down the duct, they align themselves into regular arrays. It appears that the mechanical and frictional forces at work in the duct facilitate the transformation of the soluble protein droplets into solid fibers. See Lewis, op. cit., footnote31; Kaplan, op. cit., footnote 32.

Cellulose is a polysaccharide consisting of linear glucose chains (see figure 2-4). Bacterial cellulose is synthesized in a process whereby the polymer material is extruded from the bacterial cells. Most cellulose-producing bacteria (e.g., *Acefobacter)* extrude cellulose as a ribbonlike product from a single fixed site on the cell surface. This results in the formation of a network of interlocking fibers.

Bacterial cellulose is produced under conditions of agitated fermentation. High polymer production rates occur when the growth medium contains glucose, salts, corn steep liquor, iron chelators, and various productivity enhancers. Current yields are more than 0.2 gram of cellulose per gram of glucose, and production has been demonstrated in commercial 50,000-gallon fermenters. After fermentation, the bacterial cells are destroyed during a hot caustic treatment. Bacterial cellulose is a water-insoluble material that has a very large surface area because of its large network of fibers, Bacterial fibers have roughly 200 times the surface area of fibers from wood pulp.[37] This, coupled with their ability to form hydrogen bonds, accounts for their unique interactions with water. Bacterially derived cellulose materials can absorb up to six times their weight of water, and when used as suspensions, they have pseudoplastic thickening properties. Sheets prepared from bacterial cellulose have excellent mechanical properties.

CURRENT AND POTENTIAL APPLICATIONS

Considerable progress has been made in the field of bacterial cellulose synthesis in the past few years. Bacterial cellulose is now available in limited quantities from Weyerhaeuser in the United States and Ajinomoto in Japan. The most prevalent applications of bacterial cellulose exploit its very large surface area and its ability to absorb liquids. Consequently, very low concentrations of bacterial cellulose can be used to create excellent binding, thickening, and coating agents. Because of its thickening properties, many applications in the food industry are possible. Paper that is coated with bacterial cellulose is extremely smooth and protects the underlying fibers from moisture. End uses in oil and gas recovery, mining, paints, adhesives, and cosmetics are also envisioned. This material is currently used by Sony Corporation in the production of high-end audio speaker systems because of its excellent acoustic properties.[38] In the last 10 years, at least



WEYERHAEUSER COMPANY

Cellulon® engineered bacterial cellulose fiber is a product under development at Weyerhaeuser Company. The unique reticulated network of fine fibers gives the biopolymer its thickening, binding, and coating properties. The fibers have a typical diameter of 0.1 microns (0.1 millionth of a meter), while some wood pulp fibers have a diameter of 25 to 35 microns.

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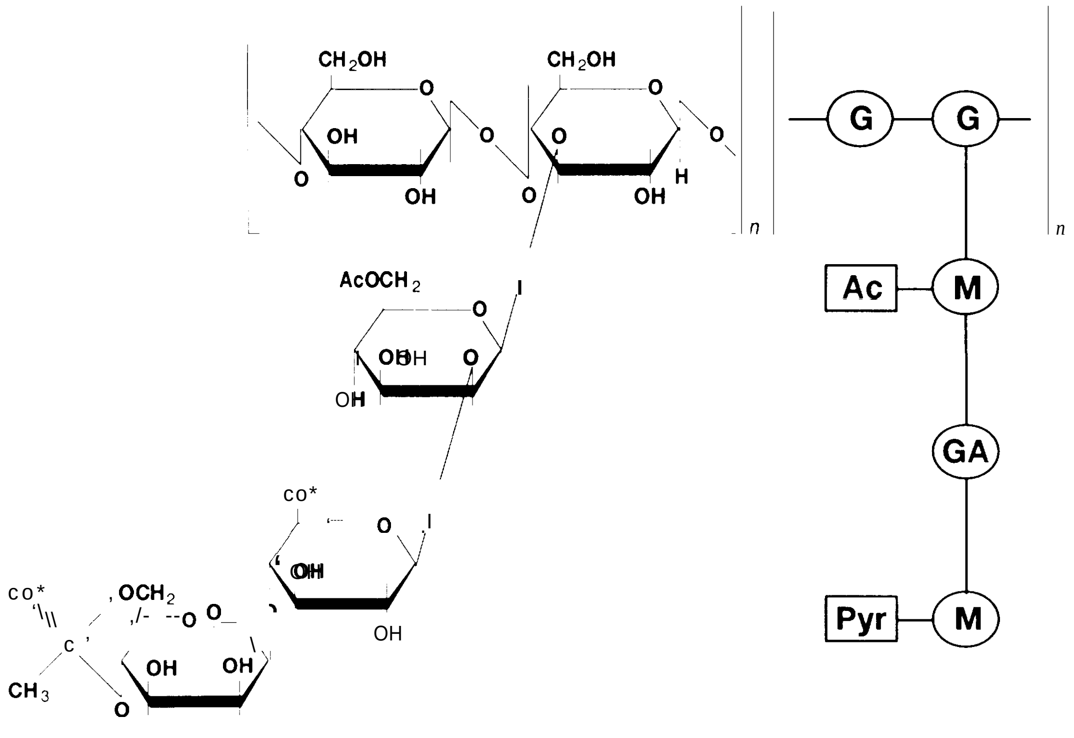
[37] Weyerhaeuser's bacterial cellulose fibers (the product is called Cellulon) have a typical diameter of 0.1 microns as opposed to some wood pulp fibers that are 25 to 35 microns across. The small fiber diameter results in an extraordinarily high surface area and is responsible for much of the fiber's functionality as a thickener and binder. A. Robert Winslow, Cellulon Fiber Business Marketing Manager, Weyerhaeuser Company, personal communication, Aug. 31, 1993.

[38] The high-fidelity headphones employ bacterial cellulose as diaphragms. The headphones retail fOr about $4,000 a set.

Figure 2-&The Structure of Xanthan Gum



CHEMICAL STRUCTURE

BLOCK STRUCTURAL REPRESENTATION

The Xanthan gum repeat unit is made of 5 sugar groups: two glucose (G) groups, two mannose (M) groups, and one gluouronic acid (GA) unit. Pyruvate (Pyr) and acetyl (Ac) units are also present in the mannose structures. The degree of pyruvate and acetate substitution varies with the specific fermentation conditions. The charged pyruvate molecule alters xanthan's electrical properties, while the acetate serves to stabilize the conformation or spatial arrangement of xanthan.

SOURCE: BioInformation Associates, Inc.., Boston, MA.

50 patents on the production and applications of bacterial cellulose have been filed. Currently, bacterial cellulose sells from $35 to $50 per pound. If the material is to be used in commodity as opposed to niche applications, production costs will have to drop. A Japanese consortium was recently formed to reduce the manufacturing costs of bacterial cellulose.[39]

XANTHAN

Xanthan gum, a complex copolymer produced by a bacterium, was one of the first commercially successful bacterial polysaccharides to be produced by fermentation. The xanthan polymer building blocks or "repeat units" contain five different sugar groups (see figure 2-5). The xanthan-producing bacterium, *Xanthomonas campestris,* is one of the frost bacterial polysaccharide

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[39] The commercial venture in bacterial cellulose technology is supported financially by the Japan Key Technology Center, a joint organization under the Ministry of International Trade and Industry and the Ministry of Post and Telecommunications, along with six private sector companies: Ajinomoto, Shimizu Construction Nikki, Mitsubishi Paper, Nikkiso, and Nakamori Vinegar. The venture is called Biopolymer Research Co., Ltd, and its principal focus will be the development of mass production techniques for cellulose that is made by fermentation *(Japan Chemical Daily,* Apr. 13, 1992).

production systems targeted for genetic engineering. Under certain conditions, genetic modification of *Xanthomonas* by using recombinant DNA technology has increased the rate of xanthan production by more than 50 percent.[40] In the future, recombinant DNA technology may enable entirely new xanthan biosynthetic pathways to be created in host organisms.

Xanthan gum is produced by large-scale fermentation of X. *campestris* using a number of different feedstocks including molasses and corn syrup. The gum is extruded from the bacteria during the polymerization process and can be recovered by alcohol precipitation following removal of the bacterial cells. For some applications such as enhanced oil recovery, the crude culture broth can be used directly following sterilization. Probably the most significant technical problem in the production of xanthan is the fact that as the polymer is produced, the fermentation medium becomes increasingly viscous. This increases the energy required for the mixing process that feeds oxygen to the bacterial cells.

CURRENT AND POTENTIAL APPLICATIONS

The unusual physical and mechanical properties of xanthan gum make it an attractive polymer for industrial and biological use. It is used extensively in both the food and the nonfood industries. Examples of industrial applications include oil recovery (provides viscosity control in drilling mud fluids), mineral ore processing (used as a biocide), paper manufacturing (used as a modifier), agriculture (acts as plant growth stimulator), pharmaceuticals (being evaluated for sustained drug release), and cosmetics (controls dust release). Food applications include gelling agents for cheese spreads, ice creams, puddings, and other deserts. More recently, xanthan has been used in the new clear-gel toothpastes. Reading the labels on many of the processed foods in the supermarket should give one a clear picture of the wide use of this material. Good examples are packet soups and many of the fat-free foods that have recently become available.

In terms of production volume, xanthan gum is the most widely used microbial polysaccharide. Worldwide production is currently in the range of 10,000 to 20,000 tons. Companies such as ADM and Merck have recently announced the expansion of their xanthan production facilities. About 60 percent of the xanthan produced is used in foods, with the remaining 40 percent used in industrial applications. Food-grade xanthan costs about $8 to $10 per pound, while non-food grades sell for about $5 per pound. Thus far, only experimental samples of genetically modified xanthan have been produced.[41]

DEXTRANS

Dextran is the generic name of a large family of microbial polysaccharides that are assembled or polymerized outside the cell by enzymes called dextran sucrases. This class of polysaccharides is composed of building blocks (monomers) of the simple sugar glucose and is stored as fuel in yeasts and bacteria. Dextrans are produced by fermentation or enzymatic conversion of the feedstock sucrose, a product of the sugar beet and sugarcane industries. Most commercial dextran

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[40] See T.J. Pollack and J. Thorne, Syntro Corp., ''Enhanced Manufacture of Xanthan Gum with Genetically Modified *Xanthomonas*,'' European Patent Application EP 287,367. CA: 9358z; and M. Yalpani, *Polysaccharide Synthesis, Modification, and Structure/Property Relations, Studies in Organic Chemistry 36* (Amsterdam: Elsevier, 1988).

[41] Two small biotechnology companies, Syntro (San Diego, CA) and Synergen (Boulder, CO), worked on genetically engineered xanthan gums in the 1980s. Neither fii was successful in marketing these xanthan products because their manufacturing costs were much higher than conventional xanthan production methods. The relatively high production costs of genetically modified polymers is a major problem that could seriously constrain biopolymer commercialization efforts. ''Conventioml wisdom' holds that biopolymer production costs must fall below $20 per pound for most niche market applications, and below $5 per pound for more general applications. Because of these manufacturing hurdles, some producers of genetically derived biopolymer compounds are focusing on low-volume, high value-added applications such as medical materials or specialized industrial adhesives. David Manyak, President, Adheron Corporation personal communication Aug. 24, 1993.

production uses the microorganism *Leuconstoc mesenteroides.* Dextran can be synthesized by using either large-scale industrial fermentors or enzymatic filtration methods. The latter approach is generally favored since it results in an enhanced dextran yield and a uniform product quality, which allows the product to be readily purified. Both of these production methods permit system conditions to be adjusted so as to control the molecular weight range of the products. This feature is an integral requirement for polysaccharide biosynthesis.

CURRENT AND POTENTIAL APPLICATIONS

Dextran polymers have a number of medical applications. Dextrans have been used for wound coverings, in surgical sutures, as blood volume expanders, to improve blood flow in capillaries in the treatment of vascular occlusion, and in the treatment of iron deficiency anemia in both humans and animals. Dextran-hemoglobin compounds may be used as blood substitutes that have oxygen delivery potential and can also function as plasma expanders. Chemically modified dextrans such as dextran sulfate have both antiulcer and anticoagulant properties. Other modified dextrans such as Sephadex@ are used extensively in the separation of biological compounds.

In the industrial area, dextrans are being incorporated into x-ray and other photographic emulsions. This results in the more economical usage of silver compounds and at the same time reduces surface gloss on photographic positives. Dextrans are used extensively in oil drilling muds to improve the ease and efficiency of oil recovery. They also have potential use in agriculture as seed dressings and soil conditioners. The protective polysaccharide coatings are found to improve germination efficiencies under suboptimal conditions.

Although many applications have been proposed for dextrans, only a small number of these have been realized and developed on a large scale. There is considerable potential for using low-molecular-weight dextrans in the biomedical industry in surgery and drug delivery systems. However, low-molecular-weight dextrans sell for about $80 per pound. As new and higher-volume applications for these materials are developed, large-scale production of dextrans may represent a major new market for the sugarcane and sugar beet industries.

PULLULAN

Pullulan is a water-soluble polysaccharide produced outside the cell by several species of yeast, most notably *Aureobasidium pullulans. Pullulan* is a linear polymer made up of monomers that contain three glucose sugars linked together (see figure 2-6).

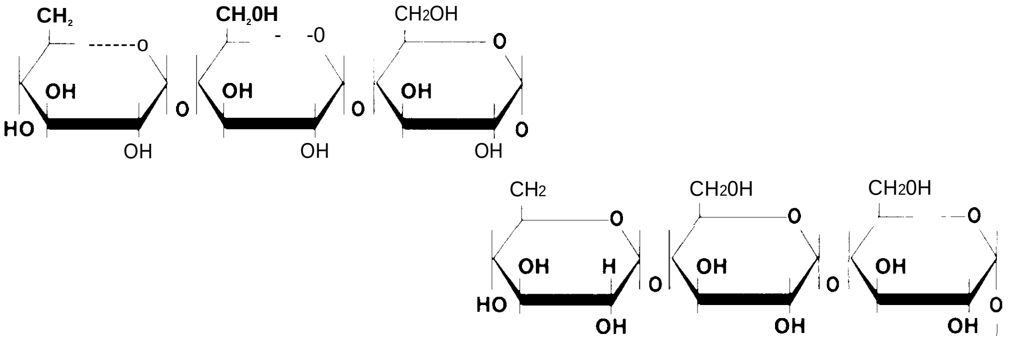
For more than a decade, a Japanese firm, Hayashibara Biochemical Laboratories, has used a simple fermentation process to produce pullulan. A number of feedstocks are used for this process, including waste streams containing simple sugars. Pullulan can be chemically modified to produce a polymer that is either less soluble or completely insoluble in water. The thermal and ionic (electrical) properties of pullulans can also be altered.

CURRENT AND POTENTIAL APPLICATIONS

Pullulan and its derivatives have a number of useful properties and consequently have many possible applications. Hayashibara Biochemical Laboratories currently sells three different pullulan grades: industrial grade ($6.50 per pound), food grade ($11 per pound), and medical grade ($15 per pound). Current efforts to increase the yields of pullulan from the fermentation of various strains of *A. pullulans* suggest lower production costs are likely in the future. Pullulan compounds are biodegradable in biologically active environments, have high heat resistance, and display a wide range of elasticities and solubilities. This versatility allows them to be utilized in many different ways.

Pullulan has many uses as an industrial plastic. It can be formed into compression moldings that resemble polystyrene or polyvinyl chloride in

Figure 2-6-The Structure of Pullulan



Pullulan is **made up of glucose sugars linked together in groups of three. The three member repeat units are connected together in a branched fashion.**

SOURCE: Office of Technology Assessment, 1993.

transparency, gloss, hardness, strength, and toughness, but is far more elastic. It decomposes above 200'C, apparently without the formation of toxic gases.[42]

A completely different application of pullulan can be found in the food industry. It can be used as a food additive, providing bulk and texture. It is tasteless, odorless, and nontoxic. It does not break down in the presence of naturally occurring digestive enzymes and therefore has no caloric content. Consequently, it can be used as a food additive in low-calorie foods and drinks, in place of starch or other fillers. In addition, pullulan inhibits fungal growth and has good moisture retention, and thus can be used as a preservative.

Pullulan can also be used as a water-soluble, edible film for the packaging of food products. It is transparent, impermeable to oxygen, and oil- and grease-resistant. Foods can be either immersed in a solution of pullulan or coated by a polymer spray. After the pullulan coating is dried, an airtight membrane is formed. The membrane can be used in the packaging of drugs and supplements, as well as oil-rich food products that are vulnerable to oxidation, such as nuts and fried foods. It is not necessary to remove the pullulan coating before eating or cooking. In the packaging of tobacco, pullulan enhances product longevity and retention of aroma. It also protects against oxidative degradation as well as attack by mold. Water insoluble coatings may be made by using the esterified or etherified forms of pullulan.

Ester and ether derivatives of pullulan have adhesive qualities similar to those of gum arabic. Viscosity and adhesive properties depend on the degree of polymerization. Modified pullulan can be used as a stationary paste that gelatinizes upon moistening. Fibers can be made from concentrated solutions of pullulan having high viscosity. The fibers have a gloss resembling rayon and a tensile strength similar to that of nylon fibers. Pullulan and its ester or ether derivatives can also be used as binders, in conjunction with other materials, for the production of nonwoven fabrics. With or without the addition of other vegetable pulps, it can be used to make paper that is suitable for printing and writing. Because it is

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[42] However, it should be noted that partial combustion of carbohydrates can produce carbon monoxide.

an antioxidant, pullulan can substitute for gum arabic in lithographic printing.

There are a plethora of other applications. Pullulan can be used as a binding agent for solid fertilizers, allowing time-released fertilization and thereby avoiding the burning of crops by controlling the release of nitrogen in the fertilizer. As a binding agent in sand molds used for metal casting, pullulan prevents the generation of dust or toxic fumes. The biopolymer can be used as a flocculating or aggregating agent for the precipitation of potash clays, uranium clays, and ferric hydroxide from slurries used in the beneficiation of mineral ores. (Currently, synthetic chemicals are primarily employed in mineral processing.) Pullulan can be used as an additive to resins and paints, where its preservative and antioxidation properties help retain color and gloss. Also, pictures or illustrations printed on pullulan film with edible ink can be transferred onto food products. In the medical area, pullulan acts as a plasma extender without undesired side effects. After metabolic turnover, it is completely excreted. Pullulan compounds can also serve as drug carriers, and can be used as medical adhesives.

Although markets for many of the applications listed here are still relatively small, with some applications only in the exploratory stage, pullulan appears to have long-term commercial potential. In sum, pullulan's many disparate uses may entitle it to become known as a biopolymer ‘‘wonder material.

GLUCANS

Glucans are, by definition, any homopolymer of the simple sugar glucose. This large group includes cellulose, pullulan, and yeast glucan. However, the term ‘‘glucan’ is commonly used to describe the glucan component of the yeast cell wall. A common source for this glucan is baker's yeast, *Saccharomyces cerevisiae*, although it is also found in a number of other sources (bacteria, fungi, lichen, and higher plants, e.g., barley).

Large supplies of inexpensive yeast are available from both the baking and the brewing (brewer's yeast) industries. Glucans are the most abundant polymers in yeast, making up approximately 12 to 14 percent of the total dry cell weight. Glucan is readily purified from yeast cells by using hot alkali treatment to remove all other cellular materials, thereby allowing recovery of the insoluble glucan material. Yeast glucan particles purified by this method contain both high molecular weight and lower molecular weight polymers.

CURRENT AND POTENTIAL APPLICATIONS

Glucans have a number of potential medical uses.[43] Glucans that are extracted from yeast cell walls are found to markedly increase the ability of some organisms (e.g., mice) to resist invading foreign bodies. Because of its action as an immunomodulator, or enhancer of the immune system, a number of studies have been performed exploring the use of glucan as an anti-infectious agent. Glucan is also effective as an antiviral agent in plants. For example, one form of glucan is very effective in protecting many species of tobacco plant against invasion by the tobacco mosaic virus and tomato black ring virus. Plants can be either injected or sprayed with the glucan polymer.

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[43] Since polysaccharides play an important role in cell adhesion and as molecular recognition elements (glycoproteins), they potentially have a number of medical applications. Recent developments in the cloning and expression of enzymes known as oligosaccharyl transferases could facilitate the creation of new carbohydrate-based pharmaceuticals. See *Chemical and Engineering News*, *“Race* Is on To Develop Sugar-Based Anti-inflammatory, Antitumor Drugs, ” Dec. 7, 1992, pp. 25-28. Apart from genetic engineering methods, some of these enzymes can also be chemically isolated. A new enzyme isolation method is being used to simplify the synthesis of novel polysaccharide compounds. Some of the new compounds have been found to prevent bacterial pneumonia in animals and are also being evaluated as anti-infective drugs. The enzymes could also be used to create polysaccharides that act as industrial coatings (e.g., as coatings on ship hulls to improve fuel efficiency and prevent marine corrosion). Stephen Roth, Neose Pharmaceuticals, personal communication, Sept. 3, 1993. Also see *Chemical and Engineering News*, “Patent Grants Broad Protection to Enzymatic Carbohydrate Synthesis,” Mar. 29, 1993, pp. 24-27.

Several studies using different tumor models in mice and rats have revealed that glucans can inhibit tumor growth. The less toxic, soluble form of glucan is effective as an antitumor agent, although it is slightly less effective than the particulate form. Many antitumor glucans are currently being used in Japan on human subjects. In the United States, one company (Alpha Beta Technology of Worcester, Massachusetts) has yeast glucans that are undergoing clinical trials as immune-stimulating agents. Another interesting property of glucans is that they are radioprotective (i.e., they appear to enhance survival by preventing death due to postirradiation infection). This enhances the survival of test animals after otherwise lethal doses of radiation.

Although glucans are being exploited principally for their antitumor, anti-infectious, and radioprotective properties, they also have nonmedical applications. Glucans resist breakdown when attacked by digestive enzymes, and thus can be used as noncaloric food thickeners. Other possible applications include use in sustained-release tablets, encapsulation of oxygen for mass transfer in fermentation reactions, and as a solid support material for chromatographic separations.

GELLAN

In addition to xanthan, pullulan, and glucan, a number of other microbial polysaccharides are being investigated for applications as thickening agents. These include succinoglycan, scleroglucan, and the three structurally related polymers rharnsan, welan, and gellan. Among these, gellan gum is the most recent microbial polysaccharide to be given Food and Drug Administration (FDA) approval for applications in food products (September 1990). Gellan is a complex polysaccharide having a four sugar repeat unit (glucose-glucuronic acid-glucose-rhamnose). It is produced by the bacterium *Pseudomonas elodea*, which is derived from plant tissue.

The production of gellan follows essentially the same fermentation process described for xanthan. The properties of gellan can be easily modified. A hot caustic treatment of gellan yields a polymer that has the desirable characteristic of low viscosity at high temperature. Cooling gellan in the presence of various cations (e.g., calcium) results in the formation of strong gels.

CURRENT AND POTENTIAL APPLICATIONS

Gellan gum represents the newest member of the microbial polysaccharides to be developed commercially. Developed and produced by the Kelco Division of Merck under the trade names Kelcogel and Gelrite, this polymer has applications in the food industry as a gelling agent in frostings, glazes, icings, jams, and jellies. GelIan currently sells for about $5 per pound.

SELECTED POLYMERS OF PLANTS AND HIGHER ORGANISMS”

Starch

Starch is the principal carbohydrate storage product of higher plants. The term starch actually refers to a class of materials with a wide range of structures and properties. Starch polymers can be extracted from corn, potatoes, rice, barley, sorghum, and wheat. The principal source of starch for industrial and food purposes is corn. In the United States, about 4.5 billion pounds of cornstarch is used annually for industrial applications.[45] Starches are mixtures of two glucan polymers, amylose and amylopectin.[46] These polymers are accumulated in plants as insoluble energy storage granules, with each granule containing a mixture of the two polymers. Plant

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[44] Higher organisms (i.e., eukaryotes) refer to all organisms except viruses, bacteria, and blue-green algae.

[45] See U.S. Congress, Office of Technology Assessment, *Agricultural Commodities* as *Industrial* Raw Materials, OTA-F-476 (Washington, DC: U.S. Government Printing Office, May 1991).

[46] Amylose is a *linear* polymer of glucose units, while amylopectin is a branched polymer of glucose units (see figure 1-3).

breeding techniques have been used to produce new strains with altered ratios of amylose to amylopectin (e.g., waxy corn contains only 0.8 percent amylose compared with natural corn, which contains 28 percent amylose, and amylomaize can contain up to 80 percent amylose). The ability to manipulate the ratio of amylose to amylopectin by strain development has drastically reduced the economic costs associated with physical separation of the two polymers. This is important because amylose and amylopectin have different properties and applications.

CURRENT AND POTENTIAL APPLICATIONS

Because of its low cost and widespread availability, starch has been incorporated into a variety of products. Chemical modification of starch polymers can lead to a number of useful derivatives. Current U.S. production of ethanol requires about 400 million bushels of corn. An additional several billion pounds of cornstarch is used for nonfuel purposes. Approximately 75 percent of the industrial cornstarch produced is transformed into adhesives for use in the paper, paperboard, and related industries. Because cornstarch can absorb up to 1,000 times its weight in moisture, it is used in disposable diapers (about 200 million pounds annually), as a treatment for burns, and in fuel filters to remove water. Cornstarch polymers are also used as thickeners, stabilizers, soil conditioners, and even road deicers.[47]

In recent years, starch has attracted considerable attention as a biodegradable additive or replacement material in traditional oil-based commodity plastics. Although a number of starch-plastic material blends have been used in different products, particularly packaging and garbage bags, there has been considerable controversy as to whether these starch-polymer composites can biodegrade.[48] Starch itself degrades readily, and is in fact one of main components of the biological food chain. When added to petroleum-derived polymers such as polyethylene, starch can in theory accelerate the disintegration or fragmentation of the synthetic polymer chains. Microbial action consumes the starch, thereby creating pores in the material, which weakens it and enables it to break apart. Many have incorrectly characterized this process as a form of biodegradation. Independent tests of polyethylene-starch blends show that starch may biodegrade, but that the overall polymer formulation will not biodegrade at any significant rate. Distintegration of polyethylene-starch blends is not the same as biodegradation. Moreover, studies indicate that degradation rates vary considerably under different temperature, oxygen, and moisture conditions. In landfills, for example, degradation rates, even for readily degradable materials, are extremely slow.[49] Under optimal conditions, breakdown of starch-plastic blends that contain less than 30 percent starch is quite slow. Some research indicates that the starch composition needs to exceed about 60 percent before significant material breakdown occurs.[50]

These problems have led to the development of a new generation of biodegradable materials that contain very high percentages of starch. Under certain conditions, starch can be combined with water and other compounds to create a resin that is somewhat similar to crystalline polystyrene. Warner-Lambert has recently introduced the NOVON@ family of polymers that contain from 40 to 98 percent starch and readily dissolve in water. The NOVON resins combine starch with other biodegradable materials, and when dis-

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[47] U.S. Congress, op. cit., footnote 45, pp. 90-99.

[48] See "Degradable Plastics Generate Controversy in Solid Waste Issues," *Chemical & Engineering News*, June 25, 1990, pp. 7-14; and "Degradable Plastics," *R&D Magazine,* March 1990, pp. 51-56.

[49] Sanitary landfills are essentially biologically inactive environments. See the testimony of Joan Harn on "Degradable Plastics and Municipal Solid Waste Management" before the Senate Committee on Governmental Affairs, July 18, 1989.

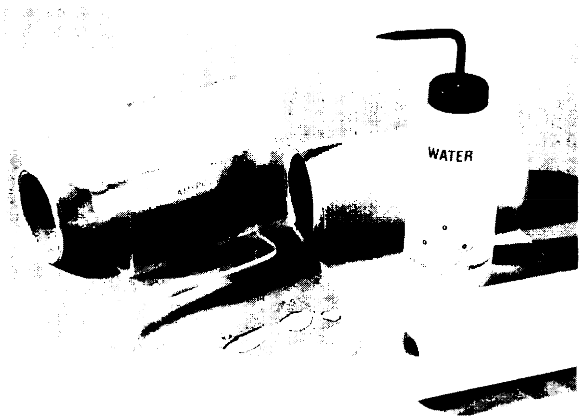
[50] U.S. Congress, Office of Technology Assessment, op. cit., footnote 45, p. 96.

posed in biologically active environments such as compost facilities and wastewater treatment systems, display degradation characteristics similar to lignocellulosic materials (e.g., leaves, woodchips, and paper).[51]

Properties of these new materials can be varied as the composition of starch and other material components change. They can replace traditional plastics used in food service, food packaging, personal health care, agricultural, and outdoor markets. Early applications of NOVON polymers include compost bags, degradable golf tees, loose-fill packaging, cutlery, pharmaceutical capsules, and agricultural mulch films. The company opened a 100-million-pound NOVON manufacturing facility in 1992. The materials are being targeted for markets where the benefits of their biodegradability can be clearly demonstrated. To fully take advantage of their environmental characteristics, however, a coordinated compost infrastructure will have to be established.[52] Although these starch-based resins cost two to four times more than commodity resins ($1.50 to $3 per pound), their novel properties might lead to the creation of new specialty markets.

Plant Cellulose

As mentioned previously, cellulose is one of the most abundant constituents of biological matter.[53] It is the principal component of plant cell walls. Among the plant cellulose, cotton fiber is the most pure, containing around 90 percent cellulose. Wood, on the other hand, consists of about 50 percent cellulose. Cellulose serves as an important material feedstock for many industries. U.S. production of cellulose fibers amounted to 485 million pounds in 1991. By adding various functional groups to the basic glucose building blocks of cellulose (see figure 2-4), a range of useful derivatives (cellulosics) can be created.



MICHIGAN BIOTECHNOLOGY INSTITUTE

New starch-based thermoplastic biopolymers such as the AMYPOL™ family of resins are water resistant and can be extruded, pelletized, and injection-molded into industrial and consumer products and films.

CURRENT AND POTENTIAL APPLICATIONS

Chemically modified plant cellulose are used in a remarkably diverse set of applications. Cellulose derivatives are used to forma variety of fibers, thickening solutions, and gels. For example, carboxymethylcellulose (CMC) is used as a thickener, binder, stabilizer, suspending agent, or flow control agent. The major markets for CMC are detergents, food, toothpaste, shampoo, skin lotions, textiles, paper, adhesives, ceramics, and latex paints. In the biotechnology area, CMC gels are used for separating molecules. Hydroxyethylcellulose (HEC) is a water-soluble compound that has major applications in the oil industry. HEC is used as a thickener in drilling fluids and as a fluid-loss agent in cementing. Hydroxypropylcellulose (HPC) has excellent surface proper-

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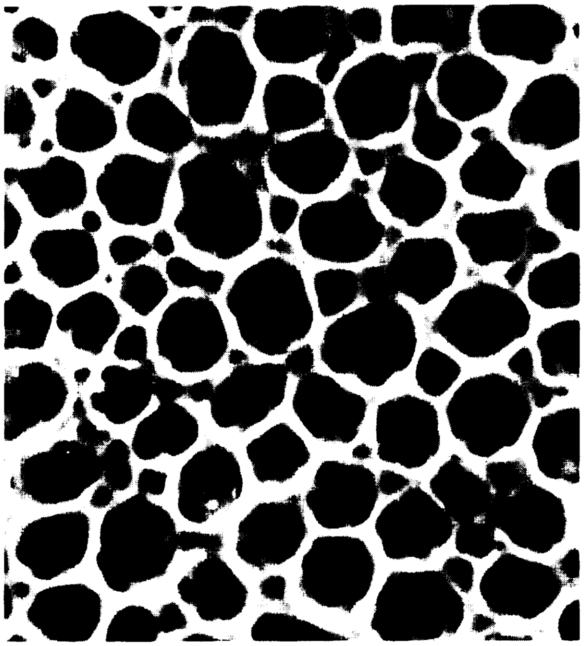
[51] The company reports that the end products of these new materials are carbon dioxide, water, and biomass, with no persistent synthetic residues. See the testimony of Ken Tracy, Vice-President of Environmental Technology, Warner-Lambert Company, before the Senate Committee on Environment and Public Works, June 5, 1991.

[52] For more on this subject, see U.S. Congress, Office of Technology Assessment, Facing *America's* Trash: *What Next for Municipal Solid Waste?* OTA-O-424 (Washington, DC: U.S. Government Printing Office, October 1989).

[53] Because of cellulose's abundance it sells for about 30 to 40 cents per pound.


TENNESSEE VALLEY AUTHORITY

A photomicrograph shows modified ethyl cellulose films magnified by a factor of 2,000. The materials are being evaluated as carriers for the controlled-release of nitrogen fertilizers.

ties and forms highly flexible films. It is used in coating pharmaceutical tablets, in molding operations, in paper coatings, and as a suspending agent in inks, cleaners, and polishes. In the medical area, hydroxypropylmethylcellulose (HPMC) has shown considerable promise as an agent for lowering blood cholesterol levels.[54]

There are many other useful derivatives. Cellulose acetate is a plastic-grade material that is widely used in packaging, particularly for blisters, skins, transparent rigid containers, and windows in folding or setup boxes.[55] In addition, cellulose acetate is used in some fabrics and as a write-on pressure-sensitive tape (e.g., for credit card receipts). Methylcellulose, created by treating cellulose fibers with methyl chloride, has excellent absorption properties and is a good thickener. It has been used in a variety of food products, including salad dressings, pie fillings, and baked goods. Nonfood applications include adhesives, agricultural chemicals, tile cements, plywood glues, printing inks, and cosmetics.

Cellulose is also receiving considerable attention as a potential feedstock for liquid fuels, particularly ethanol. By either acid or enzymatic treatment (biological enzymes break down the cellulose into its basic sugars), cellulose can be converted to fermentable glucose and then distilled to remove ethanol. Although not currently competitive with ethanol derived from corn or sugarcane, the economic attractiveness of cellulose-derived fuel could very well change with advances in biotechnology.[56]

Cellulose will no doubt continue to be a major material feedstock for a wide spectrum of industries. Future research is likely to focus on the development of new chemical derivatives and the creation of composites that combine cellulose with other biodegradable materials.

Lignin

Lignin is a polymer found in woody and herbaceous plants. Its principal function is to provide structural support in plant cell walls. Lignin consists of phenylpropane building blocks and belongs to the polyphenol family of polymers. Along with cellulose and hemicellulose, lignin is one of the three chemically distinct components occurring in plant tissue. Typically, woody and herbaceous biomass consists of 50 percent cellulose, 25 percent hemicellulose, and

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[54] See *Genetic Engineering News*, "High Molecular Weight Cellulose Derivative Shown to Lower Cholesterol," July 1993, p. 26.

[55] However, cellulose esters such as cellulose acetate are not degradable.

[56] U.S. Congress, Office of Technology Assessment, *Fueling Development: Energy Technologies for Developing Countries*, OTA-E-516 (Washington DC: U.S. Government Printing Office, April 1992), pp. 226-227.

[57] In addition t. these three principal biomass components, small amounts of other compounds can be present depending on the plant species. Common examples include fatty acids, waxes, tannins, and more specialized compounds such as terpene (used as a substitute for chlorofluorocarbons in electronics manufacturing) and taxol (a compound being explored as an anticancer dreg).

25 percent lignin.[57] Wood is a complex lingocelluolosic composite,[58] Lignin polymers are highly amorphous, three-dimensional structures that are associated with hemicellulose and play a key role in preventing decay of the lignocellulosic material. Lignin is generated in great quantities as a byproduct of wood pulping processes and consequently is relatively inexpensive. The most common commercial form of lignin is lignosulfonate, a compound derived from sulfite pulping. Higher-purity lignin can be obtained from "kraft" pulping, but this process is more costly .59

CURRENT AND POTENTIAL APPLICATIONS

At present, most of the lignin that is isolated from pulping processes is burned as an on-site fuel source. However, the material is increasingly being used in nonenergy applications.[60] Because lignin acts as a natural adhesive holding cellulose fibers together in plant cell walls, many of its commercial applications take advantage of this property. Millions of pounds of lignosulfonates are used annually for road dust control, Lignosulfonates are also employed as binding agents in molding applications and in animal feed. Lignin derivatives are beginning to be used as phenolic adhesives that can replace formaldehyde-based compounds in applications such as industrial packaging and tape.

The ionic properties of lignosulfonates and kraft lignins allow them to act as dispersants. They are being used to prevent mineral buildup in boilers and cooling towers, as thinning agents in oil drilling muds and concrete admixtures, and as dispersing agents in pesticide powders. Some major chemicals are also produced from lignin precursors. For example, vanillin, the principal ingredient in artificial vanilla, is derived from the aromatic components of lignin. In addition, chemically modified lignins are being explored for possible pharmaceutical applications. The development of specialized lignin compounds, such as electrically conducting polymers and engineering plastics, is an area of considerable research.

Chitin

Chitin, a polysaccharide, is one of the most ubiquitous polymers found in nature. It is almost as common as cellulose, and possesses many of the structural and chemical characteristics of cellulose (see figure 2-7). Chitin is an important structural component of the exoskeleton of a great number of organisms such as insects and shellfish. It also serves as a cell wall component of fungi and of numerous plankton and other small organisms in the ocean (see box 2-A for examples of other marine polysaccharides). Because of the different biological requirements of these various species, chitin is an extremely versatile natural polymer. Chitin and its most important derivative, chitosan, have a number of useful physical and chemical properties, including high strength, biodegradability, and nontoxicity.[61] Currently, the principal source of chitin is shellfish waste, but given the seasonal fluctuation of shellfish harvests, genetically engineered microbial sys-

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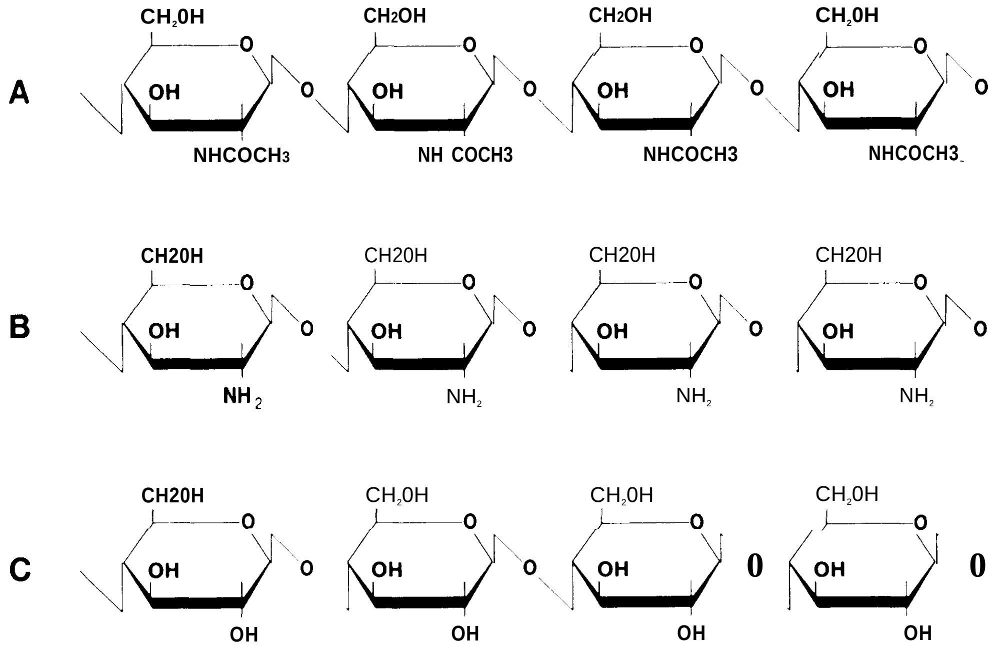
[58] Apart from their traditional use as wood products, lignocellulosic fibers are being used to create high-performance structural materials (see Roger Rowell, "Opportunities for Lignocellulosic Materials and Composites," Rowell, et al. (eds.), op. cit., footnote 1).

59 In the kraft pulping process, lignin is isolated from the rest of the woody tissue by sodium hydroxide treatment. Lignin can also be broken down by enzymatic means-a process that is cleaner than kraft or sulfite pulping, but considerably more expensive.

60 See Robert Northey, "Low-Cost Uses of Lignin,'' R. Rowell, T. Schultz, and R. Narayan (eds.), *Emerging Technologies for Materials and Chemicals from Biomass* (Washington DC: American Chemical Society, 1992).

61 When chitin acetyl groups (CH_3CO) are replaced by hydrogen to form amino groups (NH_2), chitosan is created (figure 2-7). When exposed to acids, these NH_2 groups attract hydrogen ions, forming (NH_3)+, imparting a net positive charge to the chitosan polymer. This enables chitosan to remove negatively charged compounds and contaminants from wastewater. The positively charged chitosan forms solid precipitates with these negatively charged compounds. Chitosan is one of few natural polysaccharides that has this "ionic" property (i.e., a positive charge).

Figure 2-7-Chitin and Its Polysaccharide Relatives



Although polysaccharides are made up of simple sugars, slight medications can lead to dramatically different chemical and physical properties. Shown here are the structures of: A) **chitin,** B) **chitosan, and C) cellulose. The** only difference between cellulose and chitosan is that cellulose has hydroxide (OH) groups instead of amino (NH,) groups. Chitin is identical in composition except for the acetylated amine groups (NHCH,OH).

SOURCE: Office of Technology Assessment, 1993.

terns might be used to provide a stable supply of high-grade chitin compounds.[62]

CURRENT AND POTENTIAL APPLICATIONS

The chitin family of polymers is being widely used in medicine, manufacturing, agriculture, and waste treatment. In the biomedical area, chitosan is incorporated into bandages and sutures in wound-healing treatment, because it forms a tough, water-absorbent, oxygen permeable, biocompatible film. It can be used to accelerate tissue repair and can be applied directly as an aqueous solution to treat burns. Because of its high oxygen permeability, chitosan is used as a material for contact and intraocular lenses. Chitosan has also been found to expedite blood clotting. The fact that chitin compounds are biodegradable (the human body breaks chitin down into simple carbohydrates, carbon dioxide, and water) makes them particularly appropriate for use in drug delivery systems. Chitosan carriers can release drugs slowly. This property is extremely valuable in cancer chemotherapy since the agents are often highly toxic and require long periods of time for administration. A chitosan compound is also

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[62] Certain algae, for example, produce a relatively pure form of chitin fiber that can be readily extracted and processed. However, these algae grow quite slowly. The application of biotechnology may lead to the development of fast-growing strains of algae that produce large quantities of chitin. In addition, some fungi produce chitosan directly. By modifying these strains, greater amounts of chitosan could be produced, eliminating the need for chemical treatment of chitin. See New *Scientist*, *"Life* after Death for Empty Shells, ' Feb. 9, 1991, pp. 46-48.

Box 2-A–Polymers of the Sea

As major repositories of the earth's genetic diversity, the oceans are a rich source of proteins, polysaccharides, and other polymeric compounds. Because marine organisms live in a variety of different environments-some of them extremely harsh-they have developed polymers with a wide range of properties. For example, the hard calcium carbonate shell of the abalone is held together by a glue composed of proteins and sugars, and ocean species in polar climates are able to survive extremely cold temperatures by producing antifreeze proteins. Other proteins regulate the mineralization processes involved in the creation of shells and crystals (e.g., the calcite crystals of sea urchin spines). Polysaccharides serve as structural components in crustaceans (e.g., chitin), and in a number of algal species such as kelp.

Chitin and some algal polysaccharides such as agar, alginate, and carrageenan are widely used in industry and medicine. The market for these marine polymers is several hundred million dollars annually. Agar, a major component of the cell walls of certain red algae, is used as a photographic emulsifier; a gel for cosmetics, toothpaste, and medical ointments; an inert drug carrier, a corrosion inhibitor, an adhesive, and a thickening agent in confectioneries and dairy products. Alginate is a principal structural constituent of brown algae (rockweeds and kelps), and carrageenan is extracted from red seaweed. Like agar, these two natural sugars have excellent gelling and colloidal (suspending) properties. They are used extensively in the production of ice cream and other dairy items, as well as in the textile, paper, printing, and biomedical (e.g., wound dressing and dental impression) industries.

Other marine-derived compounds are being used to formulate new drug agents, such as the anti-inflammatory compound Fucoside B. Marine organisms provide a vast range of biological processes and substances that could be genetically modified for novel medical and manufacturing purposes. The Federal investment in marine biotechnology research was about $44 million in fiscal year 1992.

SOURCES: Federal Coordinating Council for Science, Engineering, and Technology, Committee on Life Sciences and Health, Biotechnology *for the* 27st Century (Washington, DC: U.S. Government Printing Office, February 1992); Office of Technology Assessment, 1993.

being investigated as an inhibitor of the AIDS virus.[63]

The high moisture retention and film-forming characteristics of chitosan have resulted in a number of applications in the cosmetics and personal care areas. Chitosan is being utilized in hair spray, skin cream, shampoo, soap, nail polish, toothpaste, and personal hygiene products.[64] In paper manufacturing, the addition Of 1 percent chitin by weight greatly increases the strength of paper fibers, particularly when wet. Thus, chitin has been incorporated into diapers, shopping bags, and paper towels.[65] In addition to paper-chitin composites, some researchers have developed complexes of chitin and cellulose that have excellent water-resistant properties.[66] The material can be molded or made into biodegradable plastic films. Eventually, high-strength chitin polymers could be used in food packaging. One company, Technics of Japan, in trying to replicate

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[63] This research is being led by Ruth Ruprecht at Harvard University, See "Chitin Craze," Science News, vol. 144, July 31, 1993, pp. 72-74.

[64] *New* Scientist, op. cit., footnote 62.

[65] Ibid.

[66] J, Hosokawa et al., "Biodegradable Film Derived from Chitosan and Homogenized Cellulose,' *Industrial Engineering Chemical Research, vol. 29, 1990,* pp. 800-805.

the acoustic properties of crickets' wings, has even constructed audio speaker vibrators from chitosan materials.[67]

In terms of actual sales, agricultural end uses constitute the largest and most successful market for chitin and chitosan polymers. The fungi-resistant properties of chitosan have resulted in its application as a fertilizer, soil stabilizer, and seed protector. It is a yield-enhancing agent for wheat, barley, oats, peas, beans, and soybeans.[68] Thus, chitosan is used both as a seed coating and as a plant growth regulator. Chitosan is also used to recover protein wastes, particularly dairy products such as cheese whey, that are subsequently added to animal feed.

Because of its binding and ionic properties (i.e., in solution, the chitosan polymer carries a positive charge), chitosan can be used as a flocculating agent to remove heavy metals and other contaminants from wastewater.[69] Current applications in this area include treatment of sewage effluents, paper mill wastes, metal finishing residues, and radioactive wastes. In several countries, particularly Japan, chitosan is used to purify drinking water. Chitosan is also being evaluated for use in the bioremediation of toxic phenolic compounds.[70] This could greatly improve the efficiency of pharmaceutical and plastic manufacturing, by eliminating phenolic contaminates.

Chitin and its various derivatives have become important constituents in a number of diverse products and industrial processes. Pharmaceutical-grade chitin sells for about $32 per pound, while industrial grade chitin/chitosan compounds range from about $7 to $27 per pound (specific cost depends on the application). The unique chemical properties and biodegradability of this family of biopolymers presage an even wider range of applications in the future.

Hyaluronic Acid

Hyaluronic acid (HA) is a natural product that is found throughout vertebrate tissue. It also occurs as an extracellular polysaccharide in a variety of bacteria. HA plays an important physiological role in many organisms. Research indicates that HA aids tissue formation and repair, provides a protective matrix for reproductive cells, serves as a regulator in the lymphatic system, and acts as a lubricating fluid in joints. Currently, most of the HA used for research and commercial purposes is extracted from rooster combs. In the future, it is likely that this biopolymer will be produced from fermentation broths of *Streptococcus* and other bacteria.

Hyduronic acid, discovered in 1934, is a long, unbranched polysaccharide chain, composed of repeating twin sugar units. Because of the high density of negative charges along the polymer chain, HA is very hydrophilic (has a strong affinity for water) and adopts highly extended, random-coil conformations. This structure occupies a large volume relative to its mass and forms gels even at very low concentrations. It is extremely flexible and has a high viscosity.

CURRENT AND POTENTIAL APPLICATIONS

Since hyaluronic acid plays an important role in many developmental and regulatory processes of the body, it has been used principally in biomedical applications. It is an extremely attractive polymer material because it is a natural

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[67] *New Scientist*, op. cit., footnote 62.

[68] This particular chitosan product is marketed under the tradename YEA by Bentech Laboratories. An estimate of the potential market for YEA is about $160 million annually (BioInformation Associates, Inc., "Technology and Commercial Opportunities in Biodegradable Polymers," Boston, MA).

[69] See *Chemical and Engineering News*, "Chitin Removes Textile Dyes from Wastewater," July 12, 1993.

TO This application is being developed by Marizyme Corp. (Hanover, MD) in collaboration with researchers at the University Of Maryland. *Science News*, op. cit., footnote 63.

Table 2-3—Biomedical Uses of Hyaluronic Acid

Area	Application
Disease indicator	Identifies presence of liver cirrhosis, arthritis, scleroderma (tissue disease), and tumors.
Ear surgery	Scaffold material for ear surgery, healing of tympanic membrane perforations
Eye surgery	Protects corneal tissue; used In retinal reattachment, and in glaucoma surgery
Wound healing	Stimulates tissue repair.
Tendon surgery	Repair of flexor tendon lacerations, degenerative joint disease in animals
Antiadhesion	General surgery.
Scar control	General surgery

SOURCE BloInformatlon Associates, Boston, MA

product that degrades into simple sugars. Presently, its major uses are in eye surgery, treatment of arthritis, and wound-healing preparations; it is also being used in some cosmetic products. Various biomedical applications for HA are listed in table 2-3.

The unique physiochemical and structural characteristics of hyaluronic acid make it an excellent candidate for applications that require biocompatibility. However, the prices of HA are extremely high, more than $100,000 per kilogram. A recent survey estimates a total market for HA of $425 million by 1996.[71] The largest segment of this market is for surgery. The smallest segment is for cosmetics. Because of its utility in surgical treatment, the demand for hyaluronic acid is likely to expand. Some firms are now investigating genetically engineered HA produced by microbes. While the application of genetic techniques may produce HA that has greater polymer uniformity, switching to bacterial production (from rooster comb extraction) probably will not lower total production costs because of the need for expensive purification steps.[72]

POLYMERS PRODUCED BY CHEMICAL POLYMERIZATION OF BIOLOGICAL STARTING MATERIALS

Polymers that are created by the chemical polymerization of naturally occurring monomers are attracting considerable commercial interest. Although these polymers are not produced by biological systems, the fact that they are derived from basic biological building blocks confers on them many of the same properties as microbially or plant-derived biopolymers. These may include nontoxicity, biodegradability, and biocompatibility. In addition, these polymers are by definition drawing on feedstocks that are renewable. While there are several different classes of chemically synthesized biopolymers, two particular groups stand out. One is the family of polymers produced from lactic acid, a molecule used extensively in the food industry. The other is the growing ensemble of polyamino acid polymers.

Polymers Synthesized from Lactic Acid

Lactic acid (lactate) is a natural molecule that is widely employed in foods as a preservative and a flavoring agent.[73] It is also used in biomedical

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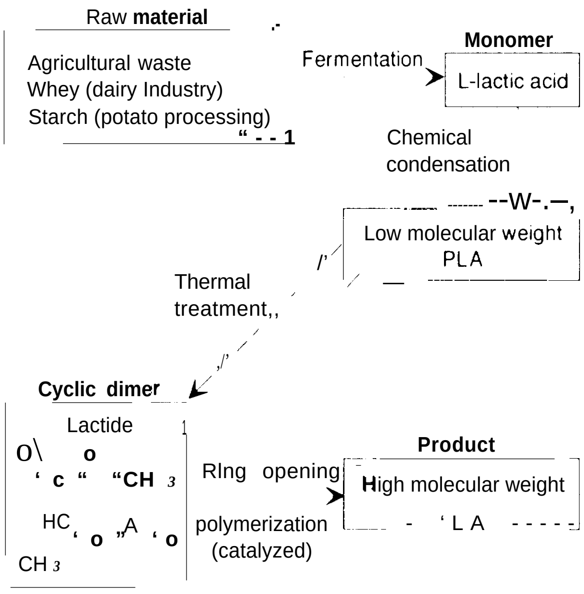
[71] See *Genetic Engineering News*, "*Complex* Carbohydrate Therapeutic Products: Tomorrow's Billion $ Market," January 1993, pp. 6-7.

[72] James Brown, Division Director, Molecular and Cellular Biosciences, National Science Foundation personal communication, July 28, 1993.

[73] Lactic acid can exist in two different forms: l-lactic acid and d-lactic acid. These two compounds are chemically identical except that they are mirror images of each other. Such mirror image structures are called stereoisomers. Because the stereoisomers have different spatial configurations, they have different reactive properties. Only the l-lactide is found in animals, whereas both the d and 1 forms of lactic acid are found in lower organisms, such as the *Lactobacilli*, a class of bacteria used for centuries in the dairy industry.

Figure 2-8-Commodity-Scale Production of Polylactide



SOURCE: BioInformation Associates, Boston, MA.

applications in intravenous and dialysis solutions. It is the main building block in the chemical synthesis of the polylactide family of polymers. This family includes polylactide homopolymers (PLA) and copolymers with glycolic acid (PLA-PGA). (Glycolic acid occurs naturally in sugarcane syrup and in the leaves of certain plants, but is generally synthesized chemically).

Lactic acid is found in blood and muscle tissue, where it is a product of the metabolic processing of glucose. Although it can be synthesized chemically, lactic acid is produced principally by the microbial fermentation of sugars such as glucose or hexose. These sugar feedstocks are drawn from agricultural (potato skins and corn) and dairy wastes. The lactic acid monomers produced by fermentation can be used to create either low or high molecular weight polylactide polymers (figure 2-8). Variation of the reaction conditions, such as temperature and choice of catalyst, provides control over the molecular weight of the polymer. In this way, the physical and chemical properties of polylactide can be adapted for different applications.

CURRENT AND POTENTIAL APPLICATIONS

Polylactide polymers are the most widely used biodegradable polyester materials. Although their principal area of application has been in the health care field, agricultural and manufacturing uses have been found as well. Polylactides are frequently used in combination with polyglycolic acid. PLA-PGA copolymers are employed as medical materials and as platforms for the sustained release of agricultural chemicals. In the industrial sector, PLA commodity polymers are being developed for use as pulping additives in paper manufacturing and as degradable packaging materials.[74] Commodity-grade polylactide sells for about $5 per pound, but manufacturers expect to bring the price down to the $2 to $3 range. Medical grade polylactide prices range from $100 to $1,000 per pound. The properties of these materials are being tailored to meet a variety of different needs.

Most applications of PLA-PGA materials have been for therapeutic purposes. Devices made of PLA-PGA copolymers have been used for the controlled release of antibiotics, anticancer and antimalarial agents, contraceptives, hormones, insulin, narcotic antagonists, and proteins.[75] The copolymers have been molded into microsphere or microcapsules, pellets, implants, and hollow fibers.

There are several other important medical applications. Polylactide sutures are widely used

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[74] Cargill, Inc. is building a manufacturing facility that will be capable of producing 10 million pounds of polylactide annually. The materials will be used as biodegradable plastics. See *Wall Street Journal*, "Concern Will Expand Move into Biodegradable Plastic," May 20, 1993, p. A7. Also, Dupont Chemical and ConAgra have formed a joint venture, EcoChem, that is developing polylactide polymers.

[75] PLA-PGA copolymers are used as drug delivery vehicles. Since protein-based drugs are quickly degraded in the body, timed-release delivery by PLA-PGA devices can decrease the rate of drug degradation.

in surgery because they degrade within the body after the incision has healed. Commercial absorbable sutures such as Vicryl@ are made of copolymers containing 90 percent PLA and 10 percent PGA. This ratio creates fibers that have excellent durability, absorbency, and tensile strength. Sutures made from PLA-PGA copolymers are stronger and absorb faster than sutures, such as Dexon@, made only of PGA. A considerable amount of ongoing research is focused on the application of PLA-PGA copolymers as sutures, clips, staples, and reinforcement materials.

PLA-PGA copolymers have also been successfully applied in experimental orthopedic surgery. Compression-molded copolymers have been used as plates or screws for the treatment of fractures and to fill in bone defects. The materials have also been used as scaffolding to facilitate the formation of new cartilage material in the body.[76] Copolymers of PLA and PGA are more useful than homopolymers of PLA and PGA because their rate of degradation can be adjusted. An advantage of using prostheses made of PLA-PGA copolymers is their biocompatibility and nontoxicity. The breadth of current research efforts suggests that the range of biomedical applications for PLA-PGA materials will expand considerably in coming years.

Polyamino Acids

Polyamino acids are an important class of synthetic polymers produced by chemical polymerization of the same amino-acid building blocks found in naturally occurring proteins.[77] Polyamino acid chains are sometimes referred to as polypeptides. Approximately 20 amino acids can be found in proteins, and from these basic building blocks a variety of homopolymers and complex copolymers have been synthesized. Because of the great chemical diversity of amino-acid monomers—anionic, cationic, hydrophobic, polar, nonpolar, thermally stable-polyamino acids can be envisioned for virtually all types of polymer applications.[78]

Currently, two principal chemical procedures are used to produce polyamino acids. One approach specifically links amino-acid monomer units together by amide bonds. These are the same type of bonds that exist in natural proteins. This particular type of chemical synthesis is a fairly complex process and is used primarily to create the high-purity materials needed for biomedical applications. In the future, this method of synthesis may be supplanted by bacterial fermentation[79] or recombinant DNA techniques. One type of bacterial species, for example, can produce poly - glutamic acid, a polypeptide that has many therapeutic uses. As highlighted earlier, recombinant DNA technology is being used to develop a new class of polymers based on spider silk (silk is a natural polyamino acid).

Polymer chains consisting of glutamic acid, aspartic acid (a component of Nutrasweet™), leucine, and valine are the polypeptides most frequently used for biomedical purposes. Lysine and methionine are also important amino-acid building blocks in polypeptide polymers.[80] Glutamic acid is the sodium salt form of monosodium

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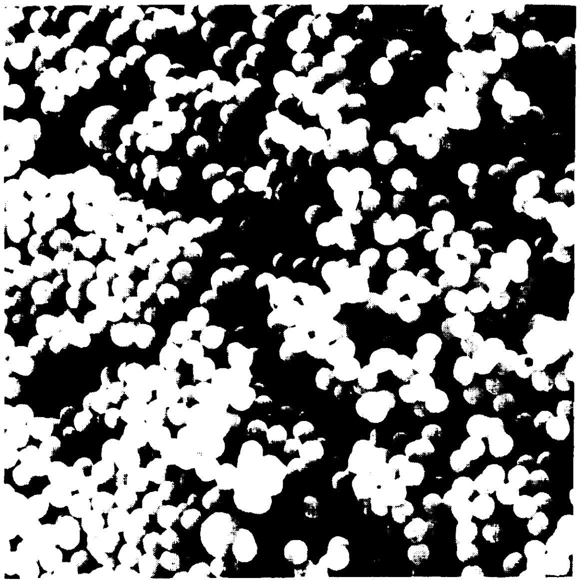
[76] See Robert Langer and Joseph Vacanti, ''Tissue Engineer ing,'' *Science*, vol. 260, May 14, 1993, pp. 920-925.

[77] As with lactic acid (footnote 73), amino acids also occur as stereoisomers; that is, as righthanded (d) or lefthanded (1) mirror image structures. One of the distinguishing features of proteins is that they are constructed only from left-handed amino acids. The synthetic polyamino acids discussed here are for the most part derived from l-amino acids. Polymers that contain only l-isomers or only d-isomers are known as homochiral structures. Like the phenomenon of DNA self-replication homochirality is a unique property of living systems. See V. Avetisov et al., "Handedness, Origin of Life, and Evolution,'' *Physics Today*, July 1991, p. 33.

[78] ''Anionic'' monomers are negatively charged; ''cationic'' monomers are positively charged.

[79] New bacterial fermentation processes that involve immobilized enzymatic reactions could potentially lower the cost of polyamino acid production.

[80] Lysine and methionine are widely used as food and animal feed additives. Methionine is produced chemically, whereas lysine is bacterially produced.



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Polyamino acid microsphere can be used to encapsulate bugs and agricultural chemicals, thereby ensuring that such active agents can be released in a controlled fashion. The microsphere shown here have a diameter of about 1.5 microns (1.5 millionth of a meter). Microsphere occur naturally and figure prominently in a theory of the origin of living cells over 3 billion years ago.

glutamate (MSG) and is a major product of the fermentation industry, with an annual production level of around 600 million pounds. A major advantage of using glutamic acid is its low cost and relative abundance. Glutamic acid and aspartic acid are hydrophilic (they have high water affinity), whereas leucine and valine are hydrophobic (low water affinity). When these hydrophilic and hydrophobic building blocks are combined, copolymers with vastly different rates of biodegradation can be created. This allows the copolymers to be used as delivery systems for a variety of different drugs. The fact that homopolymers and copolymers of these simple amino acids are nonimmunogenic (i.e., they do not produce an immune response when injected into animals) makes them particularly attractive for medical applications. Polyamino acid microspheres—spheres ranging in size from 50 nanometers (billionth of a meter) to 20 microns (millionth of a meter)--are currently being developed for oral drug administration.[81] (Polyamino acid microsphere can also be used for the controlled release of fertilizers.) In addition to drug delivery systems, polyamino acids are being investigated for use as biodegradable sutures, artificial skin, and orthopedic support materials.

A less complex chemical procedure is used to produce polyamino acids for commodity-scale industrial applications. Polypeptides are made by thermal polymerization of amino-acid building blocks at moderately high temperature (160 to 240°C), followed by a mild alkaline treatment at 60 to 80°C to open ring structures that may form. This process is not very specific and yields a polymer product in which the monomers are linked by two different types of bonds. However, the advantage of this method is that large quantities of polypeptides can be synthesized at low cost.

This approach has been used to create polyaspartate polymers from aspartic acid.[82] The polyaspartate polymers are analogues of natural proteins, particularly the aspartate-rich proteins from oyster shells that play a key role in

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[81] Protein-based drugs are extremely difficult to insert into the human body. Because proteins are easily broken down by digestive enzymes and cannot readily pass through the skin, they are currently administered by injection. By encasing protein drugs in polyamino acid materials, researchers are trying to protect the proteins long enough so that they can slip past digestive enzymes in the stomach and be absorbed into the bloodstream. This would allow oral administration of genetically administered proteins. However, in animal studies conducted thus far, only about 10 percent of an orally administered drug dose makes it to the bloodstream. One company involved in this area is Emisphere Technologies of Hawthorne, NY. See "Stand and Deliver: Getting Peptide Drugs into the Body," Science, vol. 260, May 14, 1993, pp. 912-913.

[82] Aspartic acid is fairly inexpensive as it can be produced from ammonia and maleic anhydride.

Table 2-4-Possible Applications of Industrial Polyaspartates

Water treatment	Antiscaling, anticorrosion, flocculation. Cooling towers, evaporators (for example in pulp processing), desalinators, boilers
Dispersants	Detergents, paint pigments, drilling mud, portland cement, etc
Air pollution control	Remove sulfur dioxide by preventing deposition of Insoluble sulfates
Ceramics	Promotion of crystallization of specific minerals (e g insulators)
Oilfield applications	Prevent mineralization and corrosion in well holes
Fertilizer preparation	Prevent calcification of phosphate slurries
Mineral pocessing	Antiscalants used to keep ores at an optimum size after grinding.
Textile Industry	Addition of crystallization regulators results in better fibers
Antifouling	Prevent growth of calcified organisms on marine/freshwater surfaces
Superabsorbents	Diapers
Bioplastics	At high molecular weights, polyaspartates become solid materials that may have a number of uses,
Dental treatment	Tartar control agents (toothpaste)
Biomedical devices	Prosthetic devices (heart valves), prevention of pathological calcification, microencapsulation for drug delivery, surface coatings for Implants to promote crystallization

SOURCE Steven Sikes, Department of Biological Sciences, University of South Alabama, personal communication, Aug. 11, 1993

regulating mineralization,[83] Consequently, these polymers have intrinsic antiscalant and anticorrosive properties that can prevent the buildup of mineral deposits on the surfaces of ships, cooling towers, heat exchangers, and other industrial equipment. Since the polyaspartate compounds are derived from natural precursors, they are biodegradable[84] and can be used to replace petroleum-derived polymers such as polyacrylate and polyacrylamide.[85] Because of their unique mineralization and ionic properties, there exists an enormous range of possible applications for the polyaspartate materials (see table 2-4).

At present, markets for these synthetic biopolymers are only now being identified. Nevertheless, there exist opportunities to use polyaspartates for a variety of specialized biomedical purposes, as well as a number of high-volume applications such as flocculants, dispersants, and superabsorbents. Industrial polypeptides, particularly water treatment chemicals, are likely to become commercially available in the near future.[86] It is expected that these compounds will cost from one-third to twice the price of existing synthetic chemicals such as the polyacrylamides (about $1.30 to $2 per pound).

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[83] Matrix proteins from oyster shells are powerful regulators of mineral formation. These polypeptides can inhibit mineral deposition where it is not wanted and, under certain conditions, can promote crystallization where it is needed. The formation of minerals in solution (e.g., the calcium carbonate structure of oyster shells) is a fundamental process of living and nonliving systems, It is apparent that nature has produced some extraordinary polymers to regulate the mineralization process. By investigating the properties of these natural polymers, researchers are trying to develop new biopolymers that can be tailored for a variety of industrial applications. See C. S. Sikes and A. P. Wheeler, ' 'Regulators of Biomineralization,'' *Chemtech*, October 1988, pp. 620-626.

[84] Preliminary tests of polyaspartate degradability are promising. See A.P. Wheeler and L.P. Koskan, "Large Scale Thermally Synthesized Polyaspartate as a Substitute in Polymer Applications, " *Materials Research Society Symposium Proceedings,* vol. *292,* pp. 277-283.

[85] Synthetic polymers having multiple positive or negative charges, such as polyacrylate and polyacrylamide, are widely used as water treatment additives, components in paper manufacturing, active ingredients in detergents, and tartar control agents in toothpaste, Their worldwide use amounts to billion of pounds annually, They are excellent materials in terms of their chemical activity and cost. However, they are not biodegradable.

[86] Several chemical and biotechnology companies have ongoing R&D programs involving polyaminoacids. Steve Sikes, Department Of Biological Sciences, University of South Alabama, personal communication, July 9, 1993.

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