Review Article

Polyhydroxyalkanoates: A way to sustainable development of bioplastics

Abstract

This paper reviews role of polyhydroxyalkanoates (PHAs) for sustainable development of bioplastics. PHAs are polyesters of hydroxyalkanoates with the general structural and have been investigated by biologists, chemists, engineers and other experts over the past many years. Bio-based and biodegradable plastics can form the basis for environmentally referable and sustainable alternative to current materials based exclusively on petroleum feed stocks. The result of the efforts made by scientist sought solution in form of biopolymer obtained either from microorganism or plants source and likely to replace currently used synthetic polymers as bioplastic. Applications of PHA as bioplastics, fine chemicals, implant biomaterials, and medicines have been developed. The PHA polymers promise to extend significantly the range of biomaterials as suitable alternative of plastic.

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Introduction

The research and development for polyhydroxyalkanoate (PHA) has started from the beginning of the 20th century while over the last four decades significant progress has been made in our understanding, mainly motivated by the environment friendly properties of PHA. Unlike the present petrochemical-based plastics, PHAs are produced from renewable resources. Petrochemical plastics have found widespread application in our daily life and currently in wide use being regarded as a major threat of pollution of the environment. Over the years an increased use of plastics in our routine life gave rise to the accumulation of plastic materials in our environment. Synthetic plastics are resistant to degradation, and consequently their disposal is fuelling an international attraction for the development of biodegradable polymers, compatible with our natural ecosystem. These problems have been the primary motivating factor in the research and development of PHA as a potential substitute for petrochemical-based plastics. PHAs are biocompatible as well as biodegradable, and their degradation product 3-hydroxyalkanoate is a normal mammalian metabolite.[1] PHA, a versatile class of polymers with more than 100 different monomer constituents has been obtained either from plants source such as renewable raw materials including corn, wheat, beets, sugar, potatoes, vegetable oils, other plants sources, or microorganisms. In conditions of excess nutrients, many microorganisms usually assimilate and store PHAs for future consumption.[2] A wide variety of microorganisms accumulate PHAs, a lipoidic material,[3] in the presence of an abundant carbon source, which is biochemically processed into hydroxyalkanoate (HA) units, polymerized in the presence of a key enzyme known as PHA synthase, and stored in the form of water insoluble inclusions in the cell cytoplasm. Several bacteria can accumulate PHAs up to 80% of their cell dry mass.[2-4] PHAs are biodegradable under aerobic and anaerobic conditions, and show good barrier properties against oxygen, water, and oil.

The Various Types of PHA

Depending on the types of carbon sources available and the biochemical pathways operating in the cell, microorganisms are capable of synthesizing various types of PHAs. Poly[R-3-hydroxybutyrate] (P[3HB]) is the first type of PHA identified and is the most common PHA found in nature.[5-7] Based on the molecular weight (MW), biosynthesized P[3HB] is divided into three distinct groups, i.e. low-MW P[3HB][8-10]

Roopesh Jain, Susmit Kosta, Archana Tiwari

School of Biotechnology, Rajiv Gandhi Proudyogiki Vishwavidyalaya, Gandhi Nagar, Bhopal, Madhya Pradesh, India

Address for correspondence:
Mr. Susmit Kosta,
School of Biotechnology, Rajiv Gandhi Proudyogiki Vishwavidyalaya,
Airport Bypass Road, Gandhi Nagar, Bhopal,
Madhya Pradesh - 462 036, India.
E-mail: skosta@webdunia.com

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known as complexed P[3HB] (cP[3HB]) is an ubiquitous cell constituent that exists in eubacteria, archaebacteria, and eukaryotes,[9-12] high-MW P[3HB],[6,12] and ultra-high-molecular-weight (UHMW) P[3HB].[13,14] cP[3HB] has a MW of about 12,000 Da and consists of about 120-200 3HB units.[15] On the basis of strength of their association with macromolecules, chloroform-soluble and chloroform-insoluble cP[3HB] have been identified.[16] However, high-MW P[3HB] is synthesized and accumulated in the form of water-insoluble inclusion bodies in microbial cell cytoplasm and serve as carbon and energy storage compounds for the microorganisms. The MW of this storage P[3HB] is in the range of 200,000 to 3,000,000 Da. High-MW P[3HB] was the first type of identified PHA. They are linear polyesters and their chiral centers possess only the R absolute configuration. The biosynthesized P[3HB] is thus perfectly isotactic and upon extraction from the microorganisms has a crystallinity of about 55-80% with a melting point at around 180°C.[17-19] Recently, the production of UHMW P[3HB] (MW>3,000,000) has been achieved by recombinant technology.[13] It was also noted that films prepared from this UHMW P[3HB] were completely degraded at 25°C in a natural freshwater river within 3 weeks.[20]

The P[3HB] homopolymer produced by microorganisms is rather brittle and thermally unstable than polypropylene despite having similar physical properties.[21] The brittleness is due to the formation of large crystalline domains in the form of spherulites. The formation of large spherulites is a unique character of this biologically synthesized P[3HB] possibly because of its exceptional purity. Significant improvement in the properties of P[3HB] was achieved by the incorporation of a second monomer into the 3HB sequence. Initially, it was thought that microorganisms can only synthesize the P[3HB] homopolymer but now huge family of microbial PHA[22] is known, although P[3HB] is the most common. In the 1970s, 3-hydroxyvalerate (3HV) was first identified as a member of the PHA family.[23] This eventually led to the development by Imperial Chemical Industries (ICI) for a biosynthesis process capable of producing a random copolymer of P[3HB-co-3HV] including different amounts (0-30 mol%) of 3HV units.[19,24] *Ralstonia eutropha* strain (formerly known as *Alcaligenes eutrophus*) was selected for this fermentation process. Propionic acid was added to the culture medium as a precursor carbon source giving rise to the incorporation of 3HV units. This first time enabled the production of PHA with properties that could be altered by controlling the content of the second monomer.[25,26] The development of P[3HB-co-3HV] copolymers also led to the discovery of a unique cocrystallization behavior known as isodimorphism.[2,25-28] The easiest way to control the content of 3HV units in P[3HB-co-3HV] copolymer is by changing the concentration of the carbon source that contributes to the formation of 3HV units. Recently the homopolymer of poly[R-3-hydroxyvalerate] (P[3HV]) has also been produced biologically using wild-type microorganisms such as *Rhodococcus* sp.,[29] and *Chromobacterium violaceum*, and also by using recombinant *R. eutropha* PHB-4.[30] Single crystals of these biologically synthesized P[3HV] were found to be more perfect than those of synthetic P[3HV] although they both have a square shape as opposed to the characteristic lath shape of P[3HB] crystals.[31]

Another type of PHA copolymer that shows useful physical properties is poly[3-hydroxybutyrate-co-4-hydroxybutyrate] (P[3HB-co-4HB]). Like 3HB, 4HB is a normal mammalian metabolite, which has been found in extracts of brain tissue of rat, pigeon, and man.[32] Synthetic 4HB in the form of sodium salt was first made available in the early 1960s.[33] Approximately two decades later, assimilation of this compound by *R. eutropha* to produce a random copolymer of P[3HB-co-4HB] was reported.[34,35] Subsequent studies resulted in the production of P(3HB-co-4HB) having a wide range of 4HB contents.[36-40] Other carbon sources such as 4-chlorobutyric acid, 1,4-butanediol, 1,6 hexanediol, 1,8 octanediol, 1,10-decanediol, and 1,12-dodecanediol also resulted in the incorporation of 4HB units.[41]

Unusual Polyhydroxyalkanoates

Unusual PHAs (UnPHAs) constitute a particular group of polyoxo(thio)esters related to family, tailored with uncommon monomers. Many of the uncommon monomers are incorporated only when related precursor substrates are supplied as carbon sources to the microorganisms. An interesting addition to the PHA family is the identification of a new class of sulfur-containing PHA with thioester linkages.[42] Unusual PHAs include (a) PHAs of microbial origin that have been synthesized either from natural monomers bearing different chemical functions or from chemical derivatives of the natural ones and (b) PHAs obtained either by chemical synthesis or by physical modifications of naturally occurring polymers. Regarding their chemical structure, UnPHAs are grouped in four different classes including (1) PHAs with lateral chains containing double or triple bounds or/and different functional groups (methyl, methoxy, ethoxy, acetoxy, hydroxyl, epoxy, carbonyl, cyano, phenyl, nitrophenyl, phenoxy, cyanophenoxy, benzoyl, halogen atoms, etc), (2) PHAs in which the length of the monomer participating in the oxoester linkage has been modified (the hydroxyl group to be esterified is not located at C-3), (3) polymers in which some oxoester linkages have been replaced by thioester functions (thioester-containing PHAs) and finally (4) includes those PHAs that have been manipulated chemically or physically.

PHA Biosynthesis

The rate of PHA accumulation can be increased by increasing the ratio of carbon source to nitrogen source.[43] Eventually it became evident that PHA accumulation usually occurs when cell growth is impaired due to depletion of an essential nutrient such as sulfate, ammonium, phosphate, potassium,

iron, magnesium, or oxygen.[2,44-46] PHA accumulation can also take place during active cell growth, but this ability is limited to only a few microorganisms such as *Alcaligenes latus* that can accumulate P[3HB] up to 80% of the dry cell weight without limitation of any nutrient.[47,48] An attractive feature of the microbial PHA is the ability to produce them using renewable carbon sources. The plastic materials widely in use today are synthesized from fossil fuels such as petroleum and natural gas. PHA on the other hand can be produced using renewable carbon sources such as sugars and plant oils, which is an indirect way of utilizing the atmospheric CO_2 as the carbon source. Various waste materials are also being considered as potential carbon sources for PHA production. Among them are whey,[49,50] molasses, and starch.[51,52] The carbon source available to a microorganism is one of the factors (others being the PHA synthase substrate specificity and the types of biochemical pathways available) that determine the type of PHA produced.

PHA synthases are the key enzymes of PHA biosynthesis and use the coenzyme A-thioester of (r)-hydroxy fatty acids as substrates. The two classes of PHA synthases differ in the specific use of hydroxy fatty acids of short or medium chain length resulting in PHA of the two types: (1) poly(HA SCL) from hydroxy fatty acids with short chain lengths including three to five carbon atoms, synthesized by numerous bacteria, including *Ralstonia eutropha* and *Alcaligenes latus* (PHB) and (2) poly(HA MCL) from hydroxy fatty acids with middle chain lengths including 6 to 14 carbon atoms can be made for example by *Pseudomonas putida*. A few bacteria, including *Aeromonas hydrophila* and *Thiococcus pfennigii*, synthesize copolyester from the above two types of hydroxy fatty acids. Another even large scale synthesis can be done with the help of soil organisms. The simplest and most commonly occurring form of PHA is the fermentative production of of poly-beta-hydroxybutyrate and poly-3-hydroxybutyrate [P(3HB)], which consists of 1000 to 30,000 hydroxy fatty acid monomers.

Biodegradation and Toxicity of PHA

One of the key reasons for the continued research on PHA as environment-friendly plastic is because they are biodegradable in various environments such as compost, landfill, and aquatic systems.[53-56] Water content and temperature have been found to be important along with the microbial activity in any given environment influencing biodegradation, the enzymology of the process, and the importance of polymer composition. The ability to degrade PHA is widely distributed among bacteria and fungi and depends on the secretion of specific extracellular PHA depolymerases (e-PHA depolymerases), which are carboxyesterases (EC 3.1.1.75 and EC 3.1.1.76), and on the physical state of the polymer (amorphous or crystalline). In aquatic ecosystems, even under extreme conditions (such as seasonal changes of the oxygen concentration from anoxic to oxic, low temperatures, high hydrostatic pressure, and no sunlight) plastic articles made from PHA were degraded.[57] PHA-degrading enzymes (extracellular depolymerases) are excreted by a number of bacteria and fungi in the environment (soil,[58-61] freshwater,[62] sludge,[63] seawater,[64,65] hot-springs,[66] compost,[54] air[67]). Electron microscopy analysis of PHA films revealed that degradation occurs at the surface by enzymatic hydrolysis. The degradation is therefore a function of the surface area available for microbial colonization.

Degradation is affected significantly when PHA-degrading bacteria secrete specific PHA depolymerases, which hydrolyze the polymer to water-soluble monomers or oligomeric esters and then assimilated by the microorganisms as nutrients. The following metabolisms within cells transform it to CO_2 and H_2O. Besides the environmental factors, the microstructure and properties of the PHA materials themselves can significantly affect the degradation rates.[68-70] This includes factors such as composition, crystallinity, additives, and surface area. Several kinds of extracellular depolymerases have been purified and characterized from various microorganisms.[63,65,71-73]

Importantly, degradation product components of PHAs are non-toxic in nature. Biodegradability without toxicity and thermoprocessability make PHAs attractive as biomaterials for applications in both conventional medical devices and tissue engineering. Over the past years, PHA particularly poly-3-hydroxybutyrate (PHB), copolymers of 3-hydroxybutyrate and 3-hydroxyvalerate (PHBV), poly-4-hydroxybutyrate (P4HB), copolymers of 3-hydroxybutyrate and 3-hydroxyhexanoate (PHBHHx) and its composites have been used to develop medical and surgical devices, while PHA-degraded products, including monomers and oligomers, are not harmful to the surrounding tissues and are compatible with a living body, thus are imperative as soft materials for medical purposes. Furthermore, fine particles are prepared by entrapping a hydrophilic drug on porous granules made of PHAs known to have non-toxic biodegradability and are capable of capturing the drug *in situ*.[74-77]

Applications of PHA

Microbial PHA first received widespread attention during the petroleum crisis of the 1970s as a potential substitute for petrochemical-based plastics. Extensive use of petroleum-based plastic materials has resulted in the rapid accumulation of non-degradable materials in our environment. In recent years, natural polymers are gaining much attention as the preferred materials because of environmental concerns. The biodegradability and sustainability of natural polymers are viewed as important characteristics that should be possessed by 21st century materials. Besides being a thermoplastic with properties comparable to that of polyethylene, PHA is

completely biodegradable and safe for the environment. The ability to produce PHA from renewable carbon sources also ensures a sustainable "green chemistry" process.

Investigations of PHA granules by electron microscopy after freeze-etching showed that the polymer in the granule underwent a cold drawing process indicating the plastic nature of polyester and have resulted in the development of materials having interesting physical and thermal properties as a conventional thermoplastic. In addition to its potential as plastic material, PHA is useful source of stereoregular compounds which can serve as chiral precursors for the chemical synthesis of optically active compounds, particularly in synthesis of some drugs or insect pheromones. These substances are biologically active only in the correct stereochemical configuration. The ability to further chemically modify the functional groups in these PHAs broadens their scope of application as biodegradable polymers as well as bioabsorbable materials for biomedical purposes. Numerous biomedical applications have included sutures, suture fasteners, meniscus repair devices, rivets, tacks, staples, screws (including interference screws), bone plates and bone plating systems, surgical mesh, repair patches, slings, cardiovascular patches, wound dressings, tissue engineering scaffolds, orthopedic pins, adhesion barriers, stents, guided tissue repair/regeneration devices, articular cartilage repair devices, nerve guides, tendon repair devices, atrial septal defect repair devices, pericardial patches, bulking and filling agents, vein valves, bone marrow scaffolds, meniscus regeneration devices, ligament and tendon grafts, ocular cell implants, spinal fusion cages, skin substitutes, dural substitutes, bone graft substitutes, bone dowels, wound dressings, and hemostats.[76]

PHAs are also a potential material for applications in controlled drug release systems.[74,78] The biocompatibility and biodegradability properties of PHAs make them attractive as materials for targeted drug delivery systems as a vector for targeting drugs, particularly for one of the biggest medical challenge, cancer. PHA is composed of chiral hydroxyacids that have potential as synthons for anticancer drugs, anti-HIV drugs, antibiotics, and vitamins. Importantly, it has been observed that PHA and its degradation products are not harmful to the human body, thus are expected to be imperative as soft materials for medical purposes. Some PHAs like P(4HB) can also be degraded by bacterial lipases.[79] A recent study reported the detection of lipase activities in the rat gastrointestine near the PHA implant, suggesting the involvement of lipases in the metabolism of PHA *in vivo*.[80] The 4HB units are pharmacologically active compounds, which have been used in the treatment of alcohol withdrawal syndrome[81,82] and narcolepsy. Other potential applications include the treatment of patients with chronic schizophrenia, catatonic schizophrenia, atypical psychoses, chronic brain syndrome, neurosis, drug addiction and withdrawal, Parkinson's disease and other neuropharmacological illnesses, hypertension, ischemia, circulatory collapse, radiation exposure, cancer, and myocardial infarction.

Copolyester consisting of 3-hydroxybutyrate and 3-hydroxyhexanoate (PHBHHx) is a novel PHA with better mechanical properties and biocompatibility compared with polyhydroxybutyrate (PHB) and polylactic acid (PLA). The mechanical properties of PHB can be improved when PHB is blended with PHBHHx. The high crystallization degree and rapid crystallization rate of PHB generate pores and protrusion on the PHB film surface, this coralloid surface could prohibit the attachment and growth of mammalian cells. The presence of PHBHHx in PHB strongly reduced both the crystallization degree and crystallization rate of PHB. The low crystallization degree of PHBHHx/PHB blends provided the blending films with a fairly regular and smooth surface which allowed cell attachment and growth, thus strongly improved the biocompatibility of PHB as the matrix for tissue engineering.[83]

Much work has been directed to the production of various types of PHAs for applications as commodity plastics.[84] PHAs can be easily depolymerized to a rich source of optically pure bifunctional hydroxy acids. Along with R-1,3-butanediol, it is also used to synthesize b-lactams. PHBV had received European approval for food contact use in 1996 to open opportunities in food service and packaging industry. Other practical applications of PHA includes packaging films (for food packages), bags, containers, paper coatings, insecticides, herbicides, insecticides or fertilizers, disposable items such as razors, utensils, diapers, feminine hygiene products, cosmetics containers, shampoo bottles, cups, etc. and starting material for chiral compounds.

Conclusions and Outlook

Evaluation of the performance of a product or process and their impact on the ecosystem is an important factor. Initially, interests on PHAs were mainly focused on their potential as a biodegradable thermoplastic material but there are an apparently limitless number of areas where biodegradable polymer materials may find use. These include the application of PHA mainly in the medical field where the quality and performance of materials outweigh their production costs, agriculture, automotives, and packaging. The development of PHA into a branch of bulk chemical industry addresses few important issues such as shortage of petroleum for plastic materials, reduction of CO_2 emissions, and environmental protection. Environmental accountability is constantly increasing in consequence to both consumers and industry. Biopolymers limit carbon dioxide emissions during creation, and degrade to organic matter after disposal limiting environmental burdens. The processes which hold the most promise for further development of biopolymer materials are those

which employ renewable resources identifying a process or an activity assessing energy, material used, and wastes released. Research of biodegradable materials has been the hottest topic, as society's current views on environmental responsibility make this an ideal time for further growth of biopolymers.

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