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Enzyme and metabolic engineering for the production of novel biopolymers: crossover of biological and chemical processes

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The development of synthetic biology has transformed microbes into useful factories for producing valuable polymers and/or their precursors from renewable biomass. Recent progress at the interface of chemistry and biology has enabled the production of a variety of new biopolymers with properties that substantially differ from their petroleum-derived counterparts. This review touches on recent trials and achievements in the field of biopolymer synthesis, including chemo-enzymatically synthesized aliphatic polyesters, wholly biosynthesized lactate-based polyesters, polyhydroxyalkanoates and other unusual bacterially synthesized polyesters. The expanding diversities in structure and the material properties of biopolymers are key for exploring practical applications. The enzyme and metabolic engineering approaches toward this goal are discussed by shedding light on the successful case studies.

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Introduction

The diminution of fossil resources has greatly increased the demand for the utilization of certain renewable biomass such as sugars, glycerol, and lipids, as a primary source for producing organic chemicals, including polymer materials. However, direct chemical conversion of the complex mixture of organic compounds in natural biomass into target polymers is difficult. In contrast, bioprocesses that are driven by certain highly specific enzymes along with metabolic pathway(s) are able to convert crude biomass into the desired precursors, and thus, can serve as an effective interface between biomass and the needs of the polymer industry. As shown in Figure 1, the biosynthetic systems allow for the production of a wide variety of bio-based polyesters. This review focuses on recent efforts in chemical biotechnology to create new bio-based polyesters, since the chemical processes for the production of bio-based plastics have been well documented [1,2].

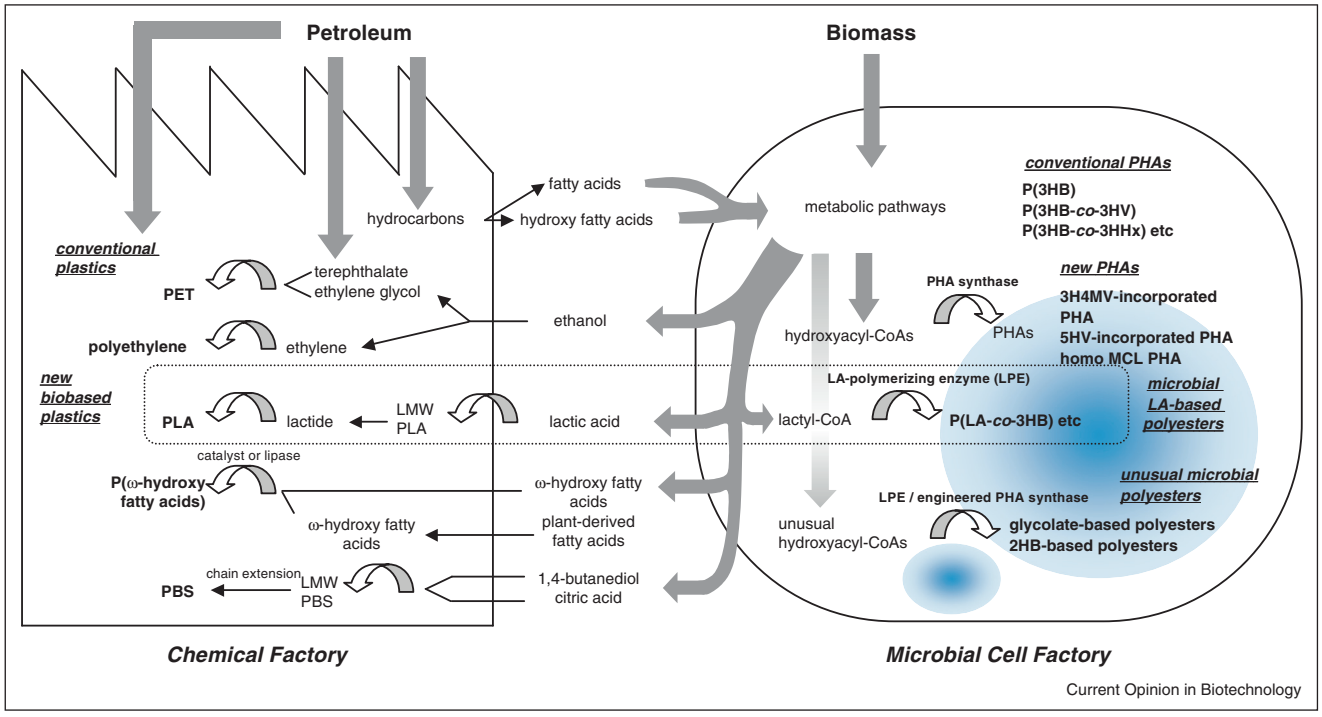
Enzymatic hydroxylation of fatty acids for the production of poly(ω-hydroxy fatty acids)

Fatty acids are an important feedstock for the production of biobased polyesters, because these carbonic acids can be polymerized into polyesters after hydroxylation (for detail see review [3•]). Poly(ω-hydroxy fatty acids) are plant oil-derived polyesters having attractive properties [4]. For example, poly(ω-hydroxytetradecanoic acid) has an elongation at break of 730% (meaning that the film can be stretched up to 730% in length before breaking off), which is similar to high-density polyethylene [5]. The ω-hydroxylation of fatty acids for obtaining monomers has typically been carried out by using chemical processes, but these processes require hazardous and energy-consuming steps that increase production cost as well as exerting an unfavorable impact on the environment. To address this problem, an engineered yeast expressing P450 was developed [6]. This strain allowed the biological synthesis of ω-hydroxy fatty acids from the corresponding fatty acids. The polymerization of the ω-hydroxy fatty acids is achieved via the synthesis of lactones followed by successive ring-opening polymerizations. For the polymerization step, lipases have been investigated as a *greener* alternative to heavy metal catalysts [7,8]. By combining these enzymatic processes, the plant oil-derived plastics were synthesized solely *via* enzymatic processes [9].

Polyhydroxyalkanoate and their applications

Polyhydroxyalkanoates are bacterial polyesters typically consisting of (*R*)-3-hydroxyalkanoates (3HAs), and a variety of monomers can be incorporated (see below) [10]. Physical properties of PHAs are tunable ranging from rigid to elastic, depending on the arrangement of monomer units in the polymer. PHAs consisting of short-chain-length [SCL, 3-hydroxybutyrate (3HB, C_4) and C_5] generally have rigid properties. SCL PHAs with ultra-high-molecular weight can be processed into strong fibers by drawing techniques [11]. In contrast, the PHA copolymers of SCL and medium-chain-length 3HA (MCL, C_6–C_{12}) are also desirable materials, because of their elasticity and toughness [12]. In 2011, the Kaneka Co. Ltd. started the commercial production of plant oil-derived P[3HB-*co*-3-hydroxyhexanoate(3HHx)], which is marketed as AONILEX®, on a production scale of approximately 1000 tons/year. Furthermore, biodegradability and biocompatibility of PHA [13] have inspired

Figure 1



Production schemes of certain bio-based polymers. The feedstock of conventional plastics is replaced with bio-based compounds. Additionally, new bio-based plastics have been developed that may serve as an alternative to conventional plastics. The microbial process may be used to synthesize various polyesters, called polyhydroxyalkanoates (PHAs). Enzyme and metabolic engineering enabled the synthesis of PHAs containing novel units. In particular, LA-based polyesters possess new and superior properties among these polymers. The thin arrows indicate the chemical conversions. The curled arrows indicate the polymerizing step. Dashed box focuses on the chemical and biological LA-based polymers. PET, poly(ethyleneterephthalate); PLA, poly(lactic acid); PBS, poly(butylene succinate); LMW, low molecular weight; PHA, polyhydroxyalkanoate; 3HB, 3-hydroxybutyrate; 3HV, 3-hydroxyvalerate; 3HHx, 3-hydroxyhexanoate; 3H4MV, 3-hydroxy-4-methylvalerate; 5HV, 5-hydroxyvalerate; MCL, medium-chain-length (C_6–C_{12}); LA, lactate; 2HB, 2-hydroxybutyrate.

studies on the use of the polymers in biomedical applications [14], for example, as scaffolds in tissue engineering [15–17]. Recently, the use of PHA for the sizing of paper has also been proposed [18].

New monomer units incorporated into PHA

An attractive feature of PHA biosynthesis is the capacity to incorporate a broad range of monomer units into the polymer chain, which enables the creation of a variety of novel polyesters. PHAs are synthesized by the polymerization of monomer substrates, which are CoA esters of hydroxyl acids, catalyzed by PHA synthase. Therefore, new PHAs can be produced if the corresponding new monomer is supplied and is also acceptable for the polymerization catalyzed by natural or engineered PHA synthases (Figure 2). For example, the non-natural substrate, 5-hydroxyvalerate (5HV), has been incorporated into PHA. A recent study reported the improved biocompatibility and biodegradability of 5HV-incorporated PHAs [19]. In addition, an MCL unit having branched side chain, 3-hydroxy-4-methyl-varelate (3H4MV), was identified as a natural constituent of PHA synthesized in *Ralstonia eutropha* [20]. The property analysis of the 3H4MV-incorporating PHA is an ongoing project.

MCL PHA homopolymers

Homopolymer of SCL-3HB, termed P(3HB), is known to be stiff and brittle. Recently, MCL PHA homopolymers have become a new class of bacterial polyesters, and consist of PHA polymers with a near homogenous composition of a single type of MCL repeating unit. The initial study found an interesting fact; the MCL PHA consisting of 86 mol% 3-hydroxyoctanoate (3HO) produced in *Pseudomonas oleovorans* was crystalline, whereas naturally synthesized MCL PHA copolymers comprised of a random mixture of MCL repeating units with different pendant group lengths were totally amorphous [21]. Thus, current studies have been directed toward the fine synthesis of homopolymers of MCL PHAs. The principle of MCL PHA homopolymers synthesis is the conversion of MCL fatty acids into their corresponding homogeneous 3HA-CoAs and successive polymerization without breaking down the fatty acids into shorter chain metabolites (Figure 3(a)). To this end, knockouts of the β-oxidation pathway have proven to be a key step