Available online at www.sciencedirect.com
ScienceDirect
Colloids and Surfaces B: Biointerfaces 60 (2007) 68–79
COLLOIDS AND SURFACES B
www.elsevier.com/locate/colsurfb

Insight in the role of bovine serum albumin for promoting the *in situ* surface growth of polyhydroxybutyrate (PHB) on patterned surfaces via enzymatic surface-initiated polymerization

Nuttawee Niamsiri [a,b,e,*], Magnus Bergkvist [b], Soazig C. Delamarre [a], Nathan C. Cady [a], Geoffrey W. Coates [c], Christopher K. Ober [d], Carl A. Batt [a,b]

[a] *Department of Food Science, Cornell University, Ithaca, NY 14853, United States*
[b] *Nanobiotechnology Center, Cornell University, Ithaca, NY 14853, United States*
[c] *Department of Chemistry and Chemical Biology, Cornell University, Ithaca, NY 14853, United States*
[d] *Department of Material Sciences, Cornell University, Ithaca, NY 14853, United States*
[e] *Department of Biotechnology, Faculty of Science, Mahidol University, Bangkok 10400, Thailand*

Received 26 April 2007; received in revised form 29 May 2007; accepted 29 May 2007
Available online 2 June 2007

Abstract

Polyhydroxyalkanoates (PHAs) are a family of aliphatic polyesters produced by a variety of microorganisms as a reserve of carbon and energy. Enzymes involved in the synthesis of PHAs can be utilized to produce polymers *in vitro*, both in bulk and on solid surfaces. Here, site-specific attachment of the key catalytic enzyme, PHA synthase, on lithographically patterned surfaces and subsequent addition of (*R*)-3-hydroxybutyryl-CoA substrate allowed us to fabricate spatially ordered polyhydroxybutyrate (PHB) polymeric structures via an *in situ* enzymatic surface-initiated polymerization (ESIP). By varying the reaction conditions, we enhanced the growth of PHB on solid surfaces and analyzed the resulting structures by fluorescence microscopy, atomic force microscopy (AFM), attenuated total reflectance Fourier transform infrared (ATR-FTIR) spectroscopy, and gel permeation chromatography (GPC). We found that stabilization of smaller PHB granule structures by an addition of bovine serum albumin (BSA) was the most important factor for a successful synthesis of a PHB layer up to 1 μm in thickness, consisting mainly of larger cluster assemblies of PHB granules that cover the entire patterned area. Immunofluorescence detection and surface contact angle analysis revealed that BSA was physically bound to the PHB polymer all through the cluster, and reduced the overall hydrophobicity of the polymer surface. Based on information obtained from AFM, kinetic measurements and various polymer characterization methods, a plausible model for roles of BSA in the enhancement of PHB formation on surfaces is discussed. Furthermore, by using biotinylated BSA conjugates, we were able to incorporate biotin groups into the PHB polymer matrix, thus generating a bioactive surface that can be used for displaying other functional biomolecules through streptavidin–biotin interaction on the PHB structures. Because of its versatility, our fabrication strategy is expected to be a useful surface modification tool for numerous biomedical and biotechnological applications.

Keywords: PHA synthase; PHB; Enzymatic surface-initiated polymerization; Bionanofabrication

1. Introduction

The desire to create more complex, functional nanoscale devices has driven scientists to redirect their attention to the development of bottom-up strategies that employ biological molecules. "Bionanofabrication" describes a fabrication process that takes advantage of the specificity and/or catalytic efficiency of biological systems to create various types of micro/nanostructures. For example, synthetic DNA has been used to align inorganic nanoparticles [1–8], and as a template to create conductive nanowires [9–14]. Certain viruses have been used to create ordered arrays of quantum dots [15], and the self-assembly properties of certain types of proteins such as ferritin [16], chaperonin [17], and bacterial S-layers [18] have been used to construct two-dimensional periodic arrays of nanoparticles. Recently, selected enzymatic polymerization processes have been employed to create polymeric micro/nanostructures

* Corresponding author at: Department of Food Science, Cornell University, Ithaca, NY 14853, United States. Tel.: +1 607 255 7902; fax: +1 607 255 8741.
E-mail address: nn22@cornell.edu (N. Niamsiri).

0927-7765/$ – see front matter © 2007 Elsevier B.V. All rights reserved.
doi:10.1016/j.colsurfb.2007.05.023

in situ on surfaces that can be further used for novel surface functionalization strategies [19–25].

Solid surfaces modified with polymeric materials have been shown to have a wide range of applications, such as protective coatings, controlled adhesion, and providing improved biocompatibility [26–34]. Among the methods for modifying solid surfaces, surface-initiated polymerization (SIP) has been intensively studied and shown to produce more robust and denser films than conventional spin coating or grafting [26,30,34]. In SIP, the reactive group that initiates the polymerization is immobilized onto the surface, and the propagating polymer chain is grown directly from the surface. SIP is particularly attractive because it is compatible with both micro- and nanolithography processes [35]. Several studies have demonstrated SIP of various monomers using techniques such as cationic, anionic, free-radical, and metal alkoxide-based initiation processes [26,30,34,36–39]. However, most of these methods often require rigorous reaction conditions and/or input of thermal energy. In addition, more environmentally friendly and less hazardous catalysts are currently desired to broaden the applications of SIP, especially in biomedical areas, where the minimization of harmful species is critical. Recently, several bio-friendly SIP approaches have emerged which involve the use of biological catalysts to synthesize biopolymer structures on solid surfaces under physiological conditions. Chow et al. [20] for example, demonstrated a SIP strategy using a biological enzyme known as terminal deoxynucleotidyl transferase to construct DNA nanostructures from oligonucleotide initiators patterned on a surface. Our laboratory has successfully developed another biologically based SIP process, enabling the bionanofabrication of a biodegradable and biocompatible polymer, polyhydroxyalkanoate (PHA), on various surfaces such as agarose, silicon, and gold [22–25].

PHAs are one of the eight classes of physiologically significant organic biopolymers along with polynucleotides, polypeptides, polysaccharides, polyisoprenoids, lignins, polyphosphates, and polythioesters. They represent a complex class of aliphatic polyesters that are synthesized by most genera of bacteria and members of the family Halobacteriaceae of the Archaea [40–44]. PHAs accumulate as non-crystalline inclusions or granules (typically 0.2–0.5 μm in diameter) when one or more nutritional elements, such as nitrogen, phosphate, sulfur, iron, oxygen, potassium or magnesium, is limiting in the presence of an excess of carbon sources. The polymer can constitute as much as 90% of the dry cell weight, with a molecular weight ranging from of 2×10^5 to 3×10^6 [40,45]. In the past 50 years, PHAs have been a subject of intense interest due to their promising use as biodegradable and biocompatible substitutes for petroleum-based thermoplastic and elastomeric materials. Because of their properties, the potential applications for PHAs span from biodegradable consumer packaging to drug delivery, surgical sutures and scaffolds in tissue engineering [46–49]. The key enzymes of PHA biosynthesis are the PHA synthases, which catalyze the conversion of (*R*)-hydroxyacyl-Coenzyme A substrates to PHA with the concomitant release of CoA [50]. There are four major classes of PHA synthases that are distinguished from one another with respect to the primary structure of the enzyme, subunit composition and substrate specificity [40,41,49]. More than 100 different types of monomers have been reported as PHA constituents [41,49], where the length and specific functional groups present in the monomers can influence the physical properties of PHAs polymer (i.e. melting point, glass transition temperature, crystallinity, and biodegradability). The most prevalent polymer of PHAs family is poly-(3-hydroxybutyrate), PHB, which has a methyl side chain.

In the past, PHA synthases from several bacterial species have been investigated, with the focus primarily on the kinetics and mechanisms of PHA granule formation in solution [51–58]. Among these, the PHA synthase from *Wautersia eutropha* H16 (64 kDa) is the most extensively studied. This enzyme has been shown to catalyze an *in vitro* polymerization of (*R*)-3-hydroxybutyryl-Coenzyme A, (3HB-CoA), to PHB (Fig. 1a) in an aqueous solution, resulting in a formation of spherical polymer granules with diameters of up to 3 μm and molecular weights up to 1.3×10^7 Da [51,52,54]. The *in vitro* polymerization of PHB displays an initial lag phase, which has been speculated to be associated with the requirement for these enzymes to form dimers in order to become active [51,59]. Several *in vitro* polymerization studies have shown that the dimerization of PHA synthase can be promoted by either reacting (pre-loading) the enzyme with substrate (priming the reaction) [60,61] or by adding multihydroxyl compounds (such as glycerol or fructose) to the enzyme solution, which promotes the conversion of monomer to dimer due to the molecular crowding effect [59,62].In previous work, we have demonstrated an *in vitro* enzymatic surface-initiated polymerization (ESIP) of PHB on agarose and homogenous silicon surfaces using immoblized N-terminal decahistidine-tagged PHA synthases from *W. eutropha* H16 (His_{10}-tagged $PhaC_{We}$) [23].

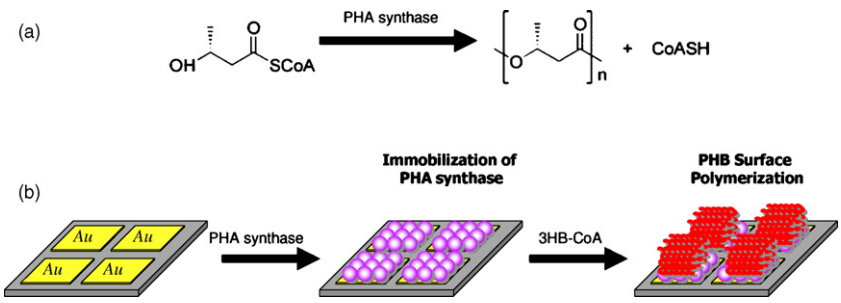

Fig. 1. (a) PHB synthesis by PHA synthase from *W. eutropha*. (b) Schematic illustration of PHB bionanofabrication on gold patterned surfaces.