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Variation in structure and properties of poly(glycerol adipate) via control of chain branching during enzymatic synthesis

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ABSTRACT

Poly (glycerol adipate) (PGA) can be produced from divinyl adipate and unprotected glycerol by an enzymatic route to generate a polymer with relatively low molar mass (12 kDa). PGA bears a pendant hydroxyl group which imparts a hydrophilic character to this water insoluble polymer. We have examined the effect of synthesis temperature on polymer characteristics through various techniques including FT-IR, ^{1}H and ^{13}C NMR, surface and thermal analysis, both to expand the data already present in the literature about this material and to understand better its properties for potential pharmaceutical applications. The use of a lipase (Novozym 435) as a catalyst suppresses cross-linking at the pendant glyceryl hydroxyl through steric hindrance at the active site, thus producing polymers with low degrees of branching (5–30%), and removes the need for any pre- or post-polymerization protection/deprotection reactions. Careful temperature control during synthesis can give polymers with reproducible molecular weights and reduced amounts of polymer branching compared to synthesis at higher temperatures. Due to the ability of the synthetic route to produce a range of structures, PGA generated by enzymatic routes may emerge as a useful biodegradable polymer platform to engineer solid dispersions or nanoparticles for healthcare applications.

1. Introduction

In recent years advances in polymer science have led to significant improvements in a plethora of fields, from electronics to healthcare, pharmacy and packaging [1,2]. In nanomedicine, polymers can be functionalized with bioactive molecules and processed in a range of shapes and sizes to address specific *in vivo* requirements. The increasing need for more sophisticated materials in various applications requires more versatility via functional macromolecules [3]. For most applications in nanomedicine, biodegradable polymers are necessary for regulatory reasons and these degradation properties can impart additional functional behavior. Poly(ortho esters) are attractive because of their inherent hydrolytic instability but poly(ortho esters) with valuable functional utility are difficult to produce because of complex monomer production, protection deprotection strategies or polymer degradation during protection/deprotection [4].

Synthetic aliphatic polyesters are widely used for biomedical, pharmaceutical and environmental applications due to their high biodegradability and low cost of production. However, the standard synthetic path for production of medical polyesters uses a metal catalyst and high temperatures (normally above 120 °C). In these conditions a wide range of collateral reactions can occur and residual toxic metals can remain entrapped in the final material. Additionally, most current metal-based catalysts lack stereo- and regio-selectivity. Over the last three decades enzymatic polymerization has been developed, becoming an important route to produce new synthetic polymers [5]. A very fertile area of research for tailored polymer synthesis is the use of enzymes in organic solvents to generate polymers with highly specific physico-chemical properties [6]. Thus lipase/esterase-catalyzed polycondensations have been developed as a suitable alternative to metal-based catalysis strategies in order to direct formation of desirable polymer structures [3,5,7–10]. Advantages of enzymatic polymerization include (a) mild reaction conditions, (b) excellent control of enantio-, chemo-, and regioselectivity, (c) ability to catalyze the ring-opening

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polymerization of macrocyclic lactones, avoiding protection/deprotection steps, (d) low toxicity and often recyclability of biocatalysts, and (e) tunable catalytic activity, with few by-products [3,5,6,9,11,12]. Hence, enzymatic polymerization is emerging as a 'green' and more selective route than traditional ones to obtain well-defined polyesters [13,14].

Enzymatic polyester synthesis can be divided into two major categories: (I) ring-opening polymerization of cyclic monomers, *i.e.* lactones or lactams [15] and (II) polycondensation of diacids with polyalcohols [16]. Enzymatic polymerizations are routinely carried out either in organic solvents or in bulk (solvent free). The enzyme is often immobilized on a solid support (such as acrylic beads, porous silica particles, etc) [11] in order to maintain activity of the biological catalyst and facilitate separation of the catalyst from the final polyester polymers. Lipases are effective catalysts for the synthesis of polyesters [6,17]. Yang et al. were the first to synthesize a family of aliphatic polyesters consisting of diesters, diols and glycolate repeat units through the copolymerization of ethyl glycolate with diethyl sebacate and 1,4-butanediol [18]. Liu et al. synthesized a series of biodegradable poly(amine-co-esters) via one step enzymatic copolymerization of diesters with C_4–C_{12} chain length and diethanolamine or its derivatives [11]. A really promising area is the enzymatic (trans)esterification of polyalcohols and di-acids (or di-esters). Iglesias et al. [19] used glycerol and adipic acid to produce hydroxylated polyesters. Partial selectivity towards the formation of 1,3 disubstituted glycerol repeating units was achieved due to the higher reactivity of lipase towards primary alcohols compared to secondary hydroxyl groups [19]. Novozym 435 (immobilized *Candida antarctica* lipase B) is the most widely used lipase enzyme utilized for the synthesis of polyesters due to its temperature and organic solvent tolerance, and high regio- chemo- and stereo-selectivity [8,20].

Kline et al. [21] first reported the synthesis of poly(glycerol adipate) (PGA) from glycerol and divinyl adipate using Novozym 435. Vinyl esters are ideal monomers for this process as the transesterification co-product, vinyl alcohol, readily tautomerizes to acetaldehyde, which is then no longer available as a substrate. This accordingly eliminates the undesired transesterification cleavage of the polyester backbone which would otherwise prevent the formation of high molecular weight polymers [16]. To avoid the more expensive vinyl ester, syntheses using unmodified adipic acid or the dimethyl ester have also been reported, but these generally require higher temperatures and carrying out the reaction under vacuum to achieve similar efficiency of polymerization [22,23]. Novozym 435 has been widely used for polymerizations of this type due to its intrinsic resistance to acetaldehyde compared to other enzymes. Work by Korupp et al. [22] has shown that optimizing the reaction conditions - temperature, pressure, enzyme concentration, reactants ratio, stirrer type, stirring rate and reaction time - enables synthesis of PGA up to a 500 g scale with ~95% monomer conversion and molecular weights (absolute molecular weight) of 2–3 kDa. By further optimizing reaction parameters such as temperature, feed ratio between monomers, and enzyme origin, Uyama et al. [24] achieved regioselective control in the lipase-catalyzed polymerization of divinyl sebacate and various triols. Yang et al. [25] compared synthesis conditions and structure of poly(oleic diacid-co-glycerol) resulting from the use of Novozym 435 or dibutyl tin oxide (DBTO) as catalysts. In the first case, a polyester with low branching degree was obtained while in the metal-catalyzed synthesis a gel was formed due to extensive cross-linking. Kallinteri et al. [26] reported the optimization of the enzymatic synthesis of PGA, mainly through control of water content, use of solvent and increased reaction time to obtain various molecular weights and demonstrated the incorporation of various amounts of different acyl substituents through subsequent modification.

PGA polymers and side-chain acylated derivatives have also been shown to self-assemble into nanoparticles with the ability to entrap a drug, dexamethasone phosphate, with increased efficiency dependent on M_w and degree of acylation of residual hydroxyls on the polymer backbone [26]. Conversely, the polar anticancer drug cytosine arabinoside showed maximum loading and slowest release from the parent unsubstituted PGA polymer with the highest molecular weight (12 kDa) [27]. Thus functionalization has the potential to provide a wide range of polymer properties which could be developed for a variety of applications. This new family of nanoparticles offers properties vital to lipophilic drug administration, such as the absence of any emulsifier or stabilizer and increased stability [28].

Both the unmodified PGA and acyl substituted PGA have been shown to have low toxicity on HL-60 and HepG2 cell lines [26], and the unmodified PGA was well tolerated in a chronic oral dosing study (No Observed Adverse Effect Level in rats determined at 1000 mg/kg/day, data not shown). Collectively these polymer properties are of great interest for drug delivery studies.

If these enzymatically synthesized polymers are to be useful for future drug delivery applications, their synthesis needs to be reproducible, convenient, able to produce polymers of high molecular weight, with well defined structures. Previous literature, particularly for polyglycerol adipate, covered a wide variety of different reaction conditions which have been employed by various authors and in addition, a wide range of temperatures up to 90 °C. The resulting polymers were often incompletely characterized. We have in this paper compared the effect of using different synthesis temperatures on the resulting physicochemical properties of PGA to establish optimum synthesis conditions. We have examined physico-chemical properties of PGA, such as Tg and contact angle. Different techniques including FT-IR, ^{1}H and ^{13}C NMR, surface and thermal analysis have been carried out to achieve a better insight of the features and behavior of these materials thereby clarifying the potential of PGA in novel drug delivery applications.

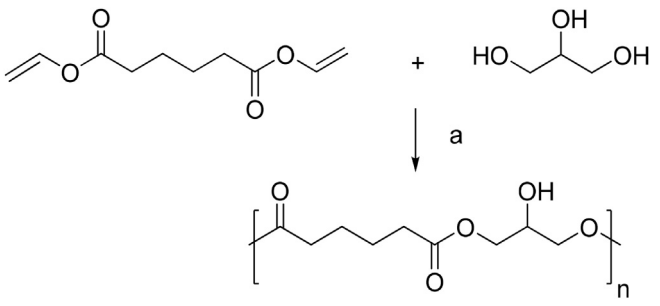
2. Experimental section

2.1. Materials

Novozym 435 lipase ([9001-62-1], derived from *C. antarctica* (>5000 U/g) and immobilized on an acrylic macroporous resin, and all solvents were purchased from Sigma–Aldrich. Divinyl adipate [4074-90-2] and HOPG (Highly Ordered Pyrolytic Graphite) were purchased from TCI America and SPI supplies, respectively. All chemicals were used as received.

2.2. Synthesis of Poly(glycerol adipate) in the range of temperature from 40 to 70 °C

PGA was synthesized (Scheme 1) by enzymatic polymerization



Scheme 1. Reagents and conditions: a. Novozym 435, 50 (60, or 70) °C, THF, 24 h.